Methodology for the Synthesis of Carbohydrate and Alkaloid Derived Natural Products

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Methodology for the synthesis of carbohydrate and alkaloid derived natural products

submitted by

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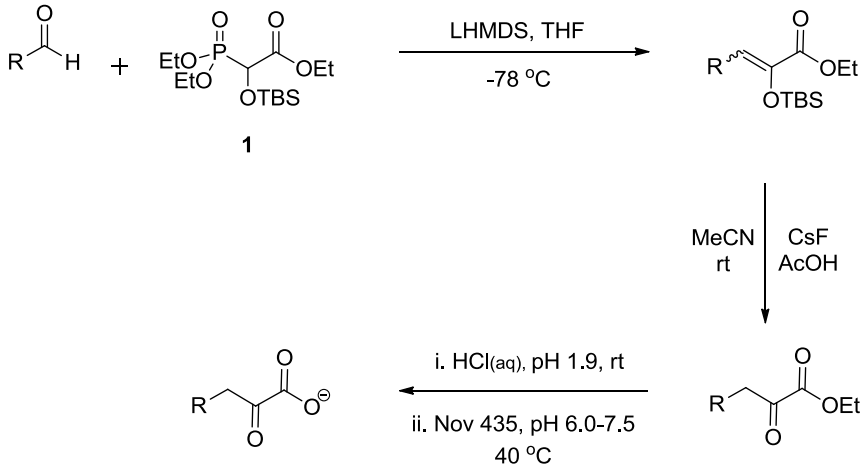
The only thing in my experience that I can compare this Ph.D to is swimming the Channel, except it has been harder. I would not have been able to reach the end without my nearest and dearest. Thanks to my parents, for their love and support, my housemate Nath who has kept me sane, my surf buddies, Eli who has been a great encourager and the best distraction from writing, and to God for his presence and mercy.

Summary

The first part of this thesis describes the synthesis, solution studies and biological evaluation of 2-keto-3-deoxy-ulosonic acids. A synthetic route was developed for 2-keto-3-deoxy-gluconate (**D-KDG**) and 2-keto-3-deoxy-galactonate (**D-KDGal**) that provided the targets *via* concise four step routes from naturally occurring sugar substrates. These routes make use of Horner-Wadsworth-Emmons reactions between the anion of ethyl 2-((*tert*-butyldimethylsilyl)oxy)-2-(dimethoxy-phosphoryl) acetate **1** with enantiopure sugar-derived aldehydes to afford silyl-enol ethers that could be globally deprotected to give the target 2-keto-3-deoxy-ulosonic acids in high purity (Scheme I). The effect of temperature on the isomeric composition of these C6-sugars was studied and they were then supplied as substrates for the directed evolution of a stereochemically promiscuous aldolase from *Sulfolobus solfataricus*, to develop mutant aldolases with high diastereoselectivity for the aldol reaction of D-glyceraldehyde **6** and pyruvate **7** to exclusively afford either **D-KDG** or **D-KDGal**.

Scheme I *Synthesis of 2-keto-3-deoxy-ulosonic acids*

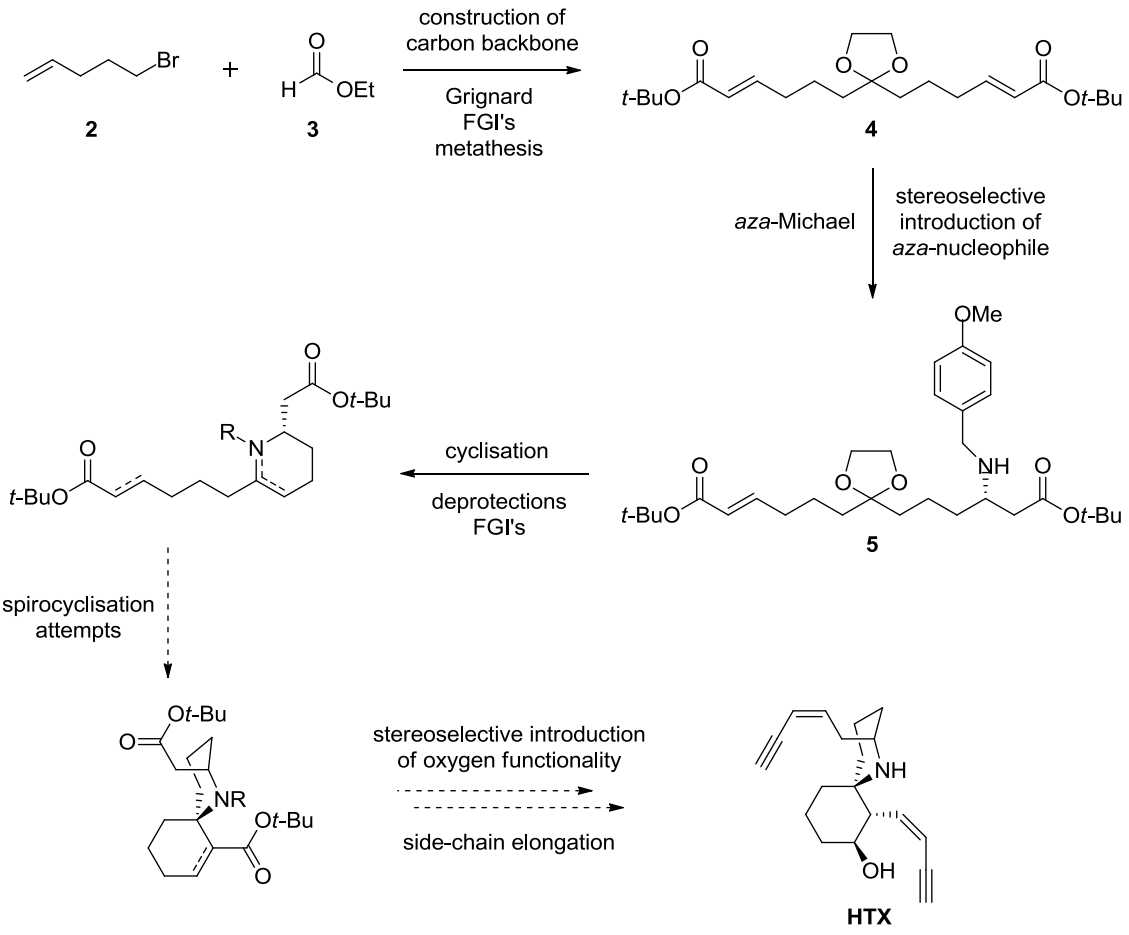


The synthetic methodology was then applied to the synthesis of enantiopure 2-keto-3-deoxy-D-xylonate (**D-KDX**) and 2-keto-3-deoxy-L-arabonate (**L-KDA**) and these C5-sugars were fully characterised by ^{1}H and ^{13}C NMR spectroscopy for the first time and had their enantiopurity confirmed by optical rotatory dispersion analysis. The kinetic parameters for **D-KDX** and **L-KDA** using KDG aldolase were determined using a modified thiobarbituric acid assay, with good catalytic efficiencies being found for each enantiomer (0.45 and

0.53 $s^{-1}mM^{-1}$). This gives a more complete understanding of the metabolism of *S. solfataricus* confirming that the archaeon uses the same KDG aldolase for the catabolism of not only the diastereotopic C6-sugars **D-KDG** and **D-KDGal** but also for the enantiomeric C5-sugars **D-KDX** and **L-KDA**.

The second part of this thesis is directed towards development of methodology for the total synthesis of histrionicotoxin (**HTX**). As part of this synthesis, an *aza*-Michael reaction was required to convert *bis*-α,β-unsaturated ester **4** into *mono*-β-amino ester **5** in high enantiomeric excess. Chapter 4 describes the development of methodology that allows the stereoselective *mono*-addition of a nitrogen nucleophile to **4**, with subsequent oxidative nitrogen deprotection to reveal the primary amino functionality. Chapter 5 then details the progress made towards **HTX** including synthesis of the acyclic carbon backbone, *aza*-Michael addition, cyclisation and attempted spirocyclisation.

Scheme II *Progress towards the total synthesis of **HTX***



Abbreviations

Å	angstroms
app.	apparent
aq.	aqueous
Ar	aryl
Bn	benzyl
bp.	Boiling point
br.	Broad
Boc	*tert*-butoxy carbonyl
Bu	butyl
d	doublet
δ	chemical shift
DCC	*N*,*N*'-dicyclohexylcarbodiimide
dd	doublet of doublets
d.e.	diastereomeric excess
DIPEA	diisopropylethylamine
DMAP	*N*-dimethylaminopyridine
DMF	dimethylformamide
DVB	divinylbenzene
e.e.	enantiomeric excess
equiv.	equivalents
ESI	electrospray ionisation
ESI-TOF	electrospray time-of-flight
Et	ethyl
Et_2O	diethylether
EtOAc	ethyl acetate
EtOH	ethanol

g	gram
g	gravities
h	hours
Hz	Hertz
HPLC	high performance liquid chromatography
HRMS	high resolution mass spectrometry
IBA	2-iodobenzoic acid
IBX	2-iodoxybenzoic acid
IR	infrared
i	*iso*
J	coupling constant
KHO	2-keto-4-hydroxyoctanoate
LDA	lithium diisopropylethylamine
LHMDS	lithium hexamethyl disilazide
m	multiplet
m	*meta*
M+	molecular ion
Me	methyl
MeOH	methanol
Mes	mesityl (1,3,5-trimethylphenyl)
min	minutes
mL	millilitre
mmol	millimole
mp.	melting point
m/*z*	mass/charge ratio
NMR	nuclear magnetic resonance
NHMDS	sodium hexamethyl disilazide
o	*ortho*

ORD	optical rotatory dispersion
oxone	potassium peroxomonosulfate
p	*para*
Ph	phenyl
ppm	parts per million
p-TSA	*para*-toluenesulfonic acid
Py	pyridine
q	quartet
rt	room temperature
s	singlet
t	triplet
TBDMS	*tert*-butyl dimethylsilyl
TCCA	trichloroisocyanuric acid
TEMPO	2,2,6,6-tetramethylpiperidin-1-yl)oxyl
TFA	trifluoroacetic acid
THF	tetrahydrofuran
TLC	thin layer chromatography
TMS	trimethylsilyl
UV	ultraviolet
ν	frequency
vis	visible

It’s not that I’m so smart, it’s just that I stay with problems longer.

— Albert Einstein

Very few people will die to save the life of someone else although perhaps for a good person someone might possibly die. But God shows his great love for us in this way: Christ died for us while we were still far away from him.

— Paul (Romans 5)

Chapter 1

1.1 Introduction

Members of the archaea family were first classified as a domain that was distinct to eukarya and bacteria in 1990,[1] and have been the centre of intense study over the last 30 years. Researchers from the Centre for Extremophile Research (CER) at Bath,[2-10] along with other groups,[11-16] have studied the archaeon *Sulfolobus solfataricus* in detail since its discovery around solfataric hot springs.[17] They have found that this archaeon metabolises C6-sugars to 2-keto-3-deoxy-ulosonic acid intermediates, before a 2-keto-3-deoxy-gluconate aldolase (KDG aldolase) catabolises these intermediates further to pyruvate and another short-chain sugar. The first part of this thesis will describe efficient syntheses of C6-2-keto-3-deoxy-ulosonic acids in diastereomerically pure form (Figure 1a) as substrates for *in vivo* directed evolution studies on KDG aldolase and enantiopure C5-2-keto-3-deoxy-ulosonic acids to allow enzyme kinetic studies to be carried out to enable a fuller understanding of the metabolic pathway of *S. solfataricus* for pentose sugars.

Figure 1a Target C6-sugars **D-KDG** and **D-KDGal**

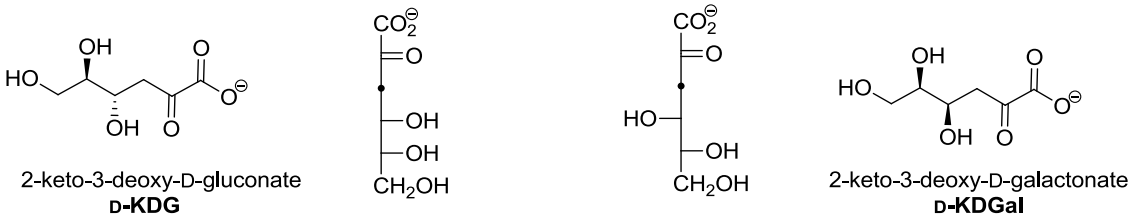
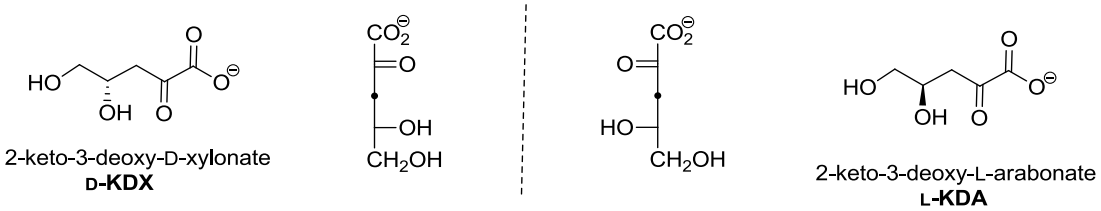


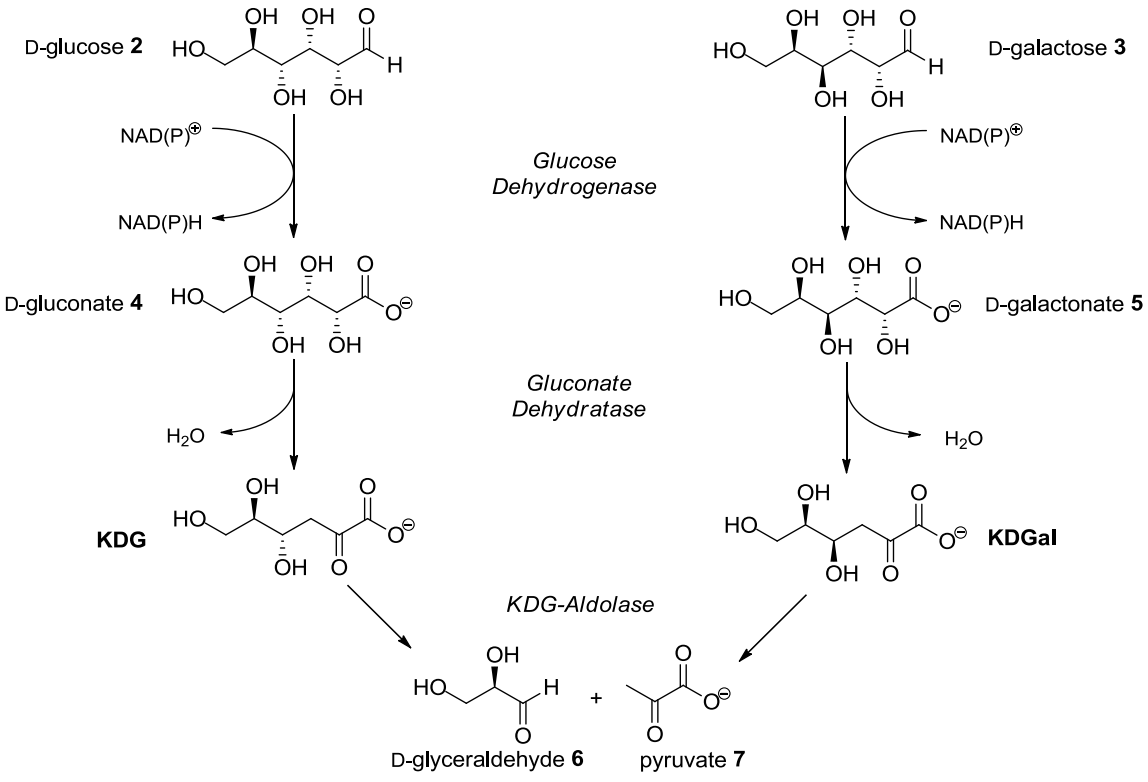
Figure 1b Target C5-sugars **D-KDX** and **L-KDA**



1.2 *Sulfolobus solfataricus*

S. solfataricus is a hyperthermophilic archaeon that grows optimally at 80-85 ˚C and pH 2-4. Whilst *S. solfataricus* grows autotrophically in its geothermal habitat by the oxidation of elemental sulphur, it is an opportunistic heterotroph,[3] able to survive on a variety of carbon sources. It metabolises glucose and galactose *via* a modified Entner-Doudoroff pathway, which is a non-phosphorylated variant of the classical pathway proceeding with no net production of ATP (Scheme 1). All three enzymes involved in the catabolism of D-glucose **2** (glucose dehydrogenase, gluconate dehydratase and KDG aldolase) are also able to catabolise D-galactose **3** to pyruvate **7** and D-glyceraldehyde **6**. The "promiscuous" nature of these enzymes is in stark contrast to the majority of bacteria and eukaryotes that catabolise D-galactose *via* the Deley Doudoroff pathway.[18] The enzyme responsible for the final step of this pathway, KDG aldolase, has been studied in particular detail because of its central role in the metabolic pathway, and the significance of carbon-carbon bond forming reactions in synthetic chemistry.[2-7] In this respect it is a potentially versatile thermostable biocatalyst for catalysing the aldol reaction of pyruvate with a range of non-phosphorylated sugars.

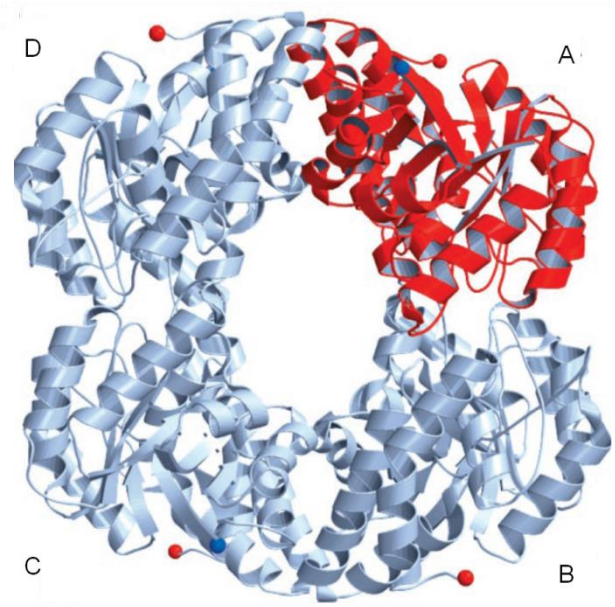
Scheme 1 *Entner-Doudoroff catabolism of D-glucose and D-galactose S. solfataricus (sugars represented in their open-chain form for stereochemical clarity)*



1.3 KDG Aldolase from *S. solfataricus*

KDG aldolase is a tetrameric enzyme, made up of four identical protein sub-units (Figure 2). It is categorized as a type-1 aldolase, since it forms a Schiff base complex between an active-site lysine residue and the donor substrate, mediating catalysis *via* transient formation of enamine intermediates. It can be efficiently expressed in *Escherichia coli* and the recombinant enzyme shows physical properties and kinetic parameters indistinguishable to that of the enzyme purified directly from *S. solfataricus* cells.

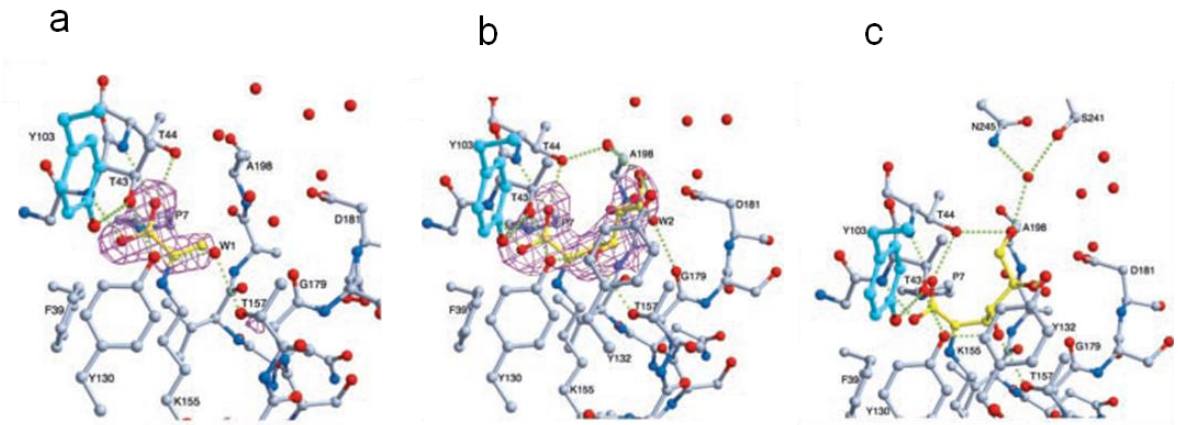
Figure 2 *KDG aldolase tetramer with monomer A highlighted in red. The blue and red spheres denote the N and C termini of each chain*[4]



Significantly for the application of *S. solfataricus* as a biocatalyst, the diastereoselectivity of the recombinant 'wild-type' enzyme with its natural substrates, **D-KDG** and **D-KDGal**, could be transformed from a ratio of 55:45 **D-KDGlu**/**D-KDGal** to values of 93% d.e. and 88% d.e. in favour of **D-KDGlu** or **D-KDGal** respectively, by mutation of key amino acid residues in the enzyme active site.[7] This rational site-directed mutagenesis approach was made possible by knowledge of the high resolution X-ray crystal structures of pyruvate **7**, **D-KDG** and **D-KDGal** covalently bound as Schiff base complexes to Lysine-155 in the active site of the enzyme (Figure 3).[4] The crystal structures revealed that a key difference in the binding of the two sugar molecules existed around the C4-C6 backbone of the molecule. The C5-OH and C6-OH of **D-KDG** were bound to a conserved network of

active-site water molecules resulting in the C4-C6 backbone presenting a relatively hydrophobic region towards threonine-157, whilst the C4-OH of **D-KDGal** was hydrogen bonded to the OH of tyrosine-130 and the OH of threonine-157. Therefore, initially threonine-157 was subjected to saturation mutagenesis with 20 single residue mutants created for all proteinogenic amino acids. A much improved d.e. for **D-KDG** was found for two of the mutants: 79% d.e. for phenylalanine-157; and 75% d.e. for cysteine-157. A double mutant combining one of these point mutations with another mutation of tyrosine-132 to valine-132 gave an impressive d.e. of 93% (Scheme 2). To shift the diastereoselectivity of the reaction towards **D-KDGal**, a series of mutants were also generated that aimed to disrupt the network of active-site water molecules that stabilised **D-KDG** formation over that of **D-KDGal**. After a first round of double mutations increased the d.e. to 72% for **D-KDGal**, a triple mutant, valine-157:Leucine-198:Glutamine-181, that was designed to create a hydrophobic pocket gave a good d.e. of 88% (Scheme 2).

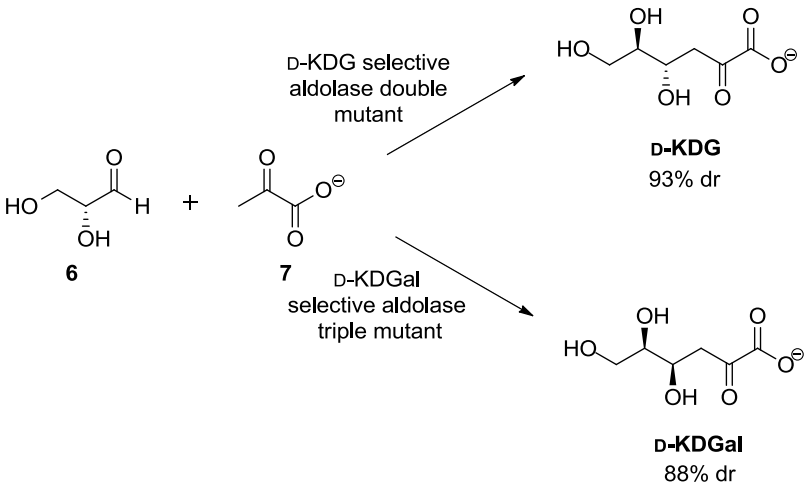
Figure 3 *S.solfataricus KDG aldolase complexed with (a) pyruvate* ***7*** *(Schiff base* $F_o - F_c$ *map contoured at 2.5 σ),(b)* ***D-KDG*** *(Schiff base* $F_o - F_c$ *map contoured at 2.5 σ) and (c)* ***D-KDGal*** *(interactions shown)*[4]



1.4 Directed Evolution of *Sulfolobus solfataricus*

A contrasting and complimentary approach was proposed for the development of mutant KDG aldolases with improved diastereoselectivity and substrate scope. This approach would involve the directed evolution of KDG aldolase by *in vivo* screening of libraries of randomly generated mutants. This approach has previously been attempted by other groups as an alternative to or compliment to site-directed mutagenesis.[19-21] The technique has generated improved mutant enzymes with amino acid mutations that are often

Scheme 2 *Best mutant aldolases for the diastereoselective aldol reaction of pyruvate* ***7*** *with* D*-glyceraldehyde* ***6***



far from the active site, with results having served to improve the understanding of subtle factors governing enzyme recognition of substrates and enzymatically catalysed stereoselective reactions.[22]

There are essentially three stages involved in directed evolution. First the generation of a library of mutant enzymes, which is normally achieved using an error prone polymerase chain reaction (ep-PCR) followed by DNA base shuffling,[23-24] and sub-cloning of the genes of the new mutant aldolases into an expression vector to generate a first generation plasmid library. Secondly the plasmid library must be transformed into bacterial cells and after an initial growth of colonies these colonies are tested for the desired activity using a high-throughput technique. The high-throughput techniques employed generally fall into two categories: (i) screening using a high-throughput assay that is preferably automated; (ii) an *in vivo* selection approach, where the bacteria colonies only survive if a mutant enzyme has the required properties. Finally, the best mutant colonies are selected, the enzyme activity is checked for the desired property and its DNA is sequenced to determine the amino acid sequence of the mutant enzyme. These three stages can be repeated in an iterative fashion until enzymes with the desired activity and kinetic parameters are identified.

For example, Wong and co-workers have published a seminal report that describes the directed evolution of D-2-keto-3-deoxy-phosphogluconate aldolase (KDPG aldolase) from

E. coli, where they aimed to improve the enzymes ability to accept non-phosphorylated substrates.[25] They generated a library of mutant enzymes using a standard ep-PCR protocol that gave an average mutation rate of 2-3 bases per gene. After sub-cloning the enzyme into an expression vector and transforming the vector in *E. coli*, the mutants were evaluated using a coupled enzyme assay in which pyruvate formed during the reaction was reduced to lactate by lactate dehydrogenase. Concomitant oxidation of NADH to NAD^+ was then monitored by absorbance at 340nm using a microtiter plate reader. The best four mutants were selected and subjected to DNA shuffling to generate 384 new mutants that were analysed for activity using this high-thoughput screening methodology. Again the best two mutants (KA2 and KA3) were selected and subjected to a further round of ep-PCR to generate 1024 variants, from which high-throughput screening identified two improved mutants (KA3.1 and KA3.3). The best mutant enzyme from the three rounds of mutation had greatly improved kinetic parameters for the *retro*-aldol reaction of **D-KDG**, exhibiting a 25-fold decrease in K_m and a 3-fold increase in k_{cat} (Table 1).

Table 1 *Kinetic parameters for **D-KDG** cleavage by recombinant wild-type KDPG aldolase, and for the best mutants generated via directed evolution*

Entry	Enzyme	K_m (mM)	k_{cat} (s^{-1})	k_{cat}/K_m ($mM^{-1}s^{-1}$)
1	Wild Type	284.9	4.6	0.016
2	KA2	14.3	8.3	0.58
3	KA3	12.8	13.9	1.09
4	KA3.1	2.4	0.05	0.021
5	KA3.2	12.1	5.2	0.43

These types of directed evolution techniques have been employed by other groups and used to alter enzyme substrate specificity,[26] thermal stability,[27] resistance to organic solvents[28] and stereoselectivity.[29] Recently Toone and co-workers have presented their work on the directed evolution of KDPG aldolases from *E. coli* and *Thermatoga maritima*.[30-31] They aimed to increase the enzymes tolerance for unfunctionalised non-polar aldehydes, choosing as a test substrate 2-keto-4-hydroxyoctanoate (KHO), whose catalytic efficiency was decreased by 10^4-10^6-fold when compared to KDPG. Again aldolase mutants were generated by ep-PCR and these were transformed into an auxotrophic *E. coli* PB25 cell line to allow an *in vivo* selection approach. The *E. coli* PB25 auxotrophs had both pyruvate kinase gene sequences interrupted, which ensured that only colonies that were supplemented with exogenous pyruvate could survive. Colony selection was therefore based on rescue of those mutant auxotrophs that could catalyse

retro-aldol cleavage of KHO when it was added as a supplement to the growth media. Based on transformation controls, Toone estimated that $5x10^7$ variants were screened when these variants were plated. Plasmid DNA was recovered and sequenced for the colonies that were surviving after 120 hours, and the kinetic parameters for KHO cleavage for each new mutant evaluated (Table 2). All the mutants, entries 2-8, showed increased catalytic efficiency, but this was mainly due to a reduction in the value of K_m with only a small increase in the turn-over number observed for three of the mutants (entries 3, 6 and 8). The authors reasoned that the intracellular concentrations of KHO must have been low, which had led to the main selection pressure being to reduce the value of K_m. They concluded that for their *in vivo* selection to be more successful the intracellular concentration of substrate must be increased or the aldolase enzyme should be localised to the periplasm where the concentration of small molecules like KDO is generally unregulated by the cell.

Table 2 *Kinetic parameters for KDPG aldolase mutants for the retro-aldol cleavage of KHO*

Entry	Enzyme	K_m (mM)	K_{cat} (s^{-1})	k_{cat}/K_m (mM^{-1}/s^{-1})
1	Wild Type	200	0.40	2.2
2	Mutant 1	9	0.07	9.3
3	Mutant 2	90	0.50	5.5
4	Mutant 3	20	0.14	7
5	Mutant 4	23	0.36	16
6	Mutant 5	14	0.75	54
7	Mutant 6	8	0.13	17
8	Mutant 7	49	1.2	25

For the directed evolution of the KDG aldolase from *S. solfataricus*, ep-PCR work in the CER has resulted in the generation of libraries of 10^4–10^5 mutants as potential biocatalysts for screening. These mutant aldolases have been expressed within a pyruvate kinase deficient *E. coli* auxotroph that is unable to survive without the addition of exogenous pyruvate to the growth media.[30,32] This enables a high-thoughput methodology to be used to assess each mutants' activity towards aldol substrates using a life/death selection assay. It is intended that the transformed cells will be cultured on media supplemented with pyruvate, with colonies that grow being transformed onto two separate daughter plates. Each daughter plate would contain minimal media with sufficient supplements, nucleotide bases and minerals for the auxotrophs to survive, with

the proviso that pyruvate is available to them.[31] The daughter plates will have one additional supplement: one will be presented with **D-KDG**; and the other daughter plate will be presented with **D-KDGal**. By comparing the growth of colonies on each plate over time, mutant KDG aldolases would be identified that are only capable of surviving on one of the daughter plates, thus identifying a mutant with *retro*-aldol cleavage activity for only one of the sugar diastereomers. The principle of microscopic reversibility then predicts that this mutant KDG aldolase would catalyse a highly stereoselective aldol condensation reaction. This would then be confirmed by identifying and purifying a diastereoselective mutant colony and screening it for the aldol condensation of pyruvate **7** with D-glyceraldehyde **6**.

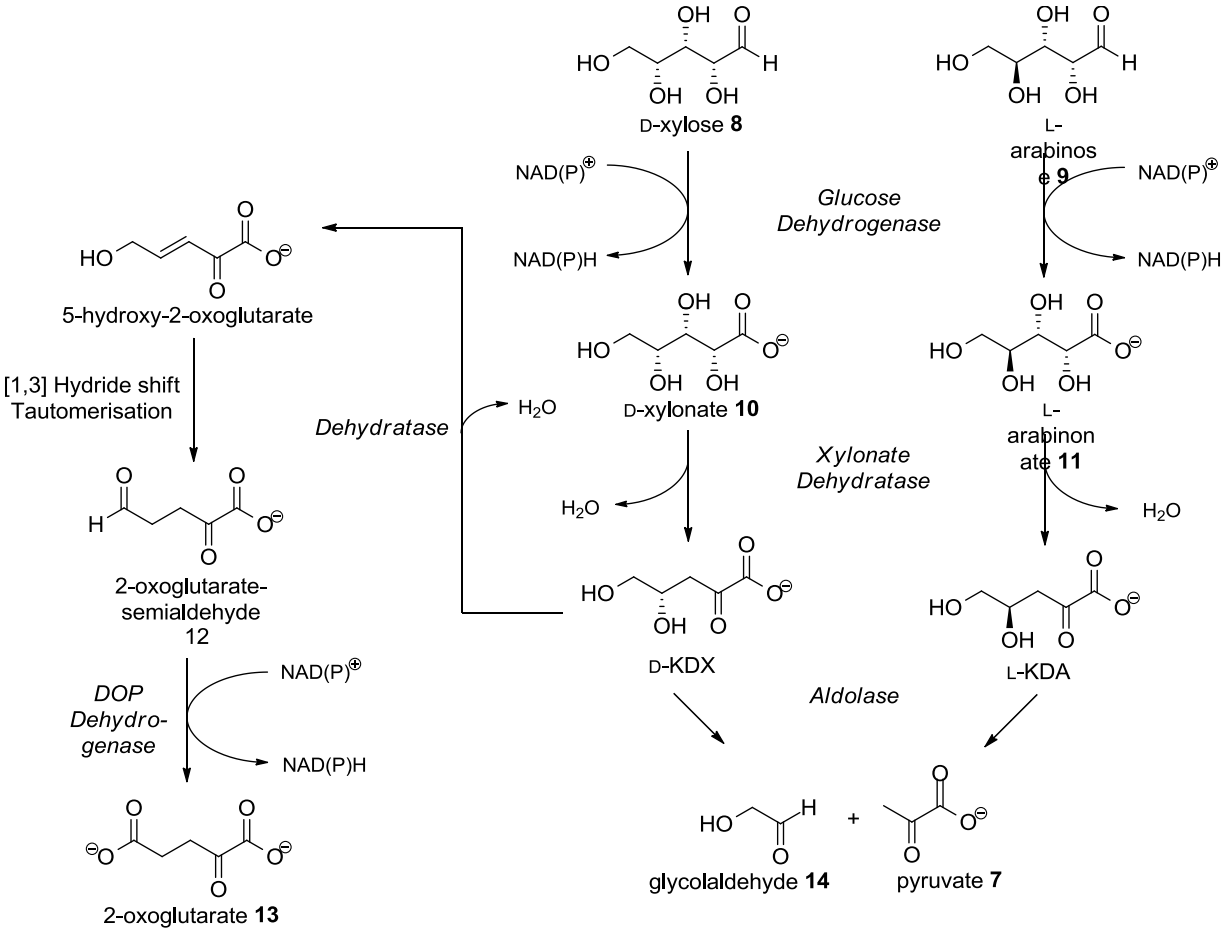
These directed evolution studies of KDG aldolase could only be carried out in an efficient manner if gram quantities of enantiopure **D-KDG** and **D-KDGal** in >99% e.e. were available as substrates for preparation of the agar medium for each of the daughter plates. Therefore, one of the major aims of my research program was to develop efficient methodology for the synthesis of **D-KDG** and **D-KGal**.

1.5 Studies of *S. solfataricus* Metabolism of C5-Substrates

Recently, Danson and co-workers have published details of the catabolic pathway that *S. solfataricus* employs for metabolism of the five carbon sugars D-xylose **8** and L-arabinose **9** (Scheme 3), which along with D-glucose and D-galactose are the four most commonly occurring sugars in nature.[6] Glucose dehydrogenase, that is responsible for the oxidation of D-glucose **2** to D-gluconate **4**, also catalyses the oxidation of D-xylose **8** and L-arabinose **9**. Conversely, gluconate dehydratase that dehydrates D-gluconate **4** to **D-KDG** has no activity with D-xylonate **10** or L-arabinonate **11**. Instead a specific dehydratase dehydrates the C5 sugars to **D-KDX** and **L-KDA**. There are then two competing catabolic pathways; approximately half of the catabolised sugar is converted to 2-oxoglutarate **13**,[11] whilst the remainder is catabolised to pyruvate **7** and glycolaldehyde **14**. KDG aldolase was shown to catalyse the aldol condensation reaction of glycolaldehyde **14** and pyruvate **7** and so could potentially be responsible for the catabolism of both **D-KDX** and **L-KDA**, but enantiomerically pure **D-KDX** and **L-KDA** were not commercially available to enable the direct measurement of their kinetic parameters with KDG aldolase. Therefore, a coupled enzyme assay was conducted to establish if KDG aldolase could catabolise both C5-sugars. This involved separate incubation of D-xylonate **10** and L-arabinonate **11** with semi-purified D-xylonate dehydratase to give crude samples of **D-KDX** and **L-KDA**. These sugars were then cleaved *via* separate incubation with KDG aldolase to give pyruvate **7**

and glycolaldehyde **14** in both cases, suggesting that both substrates could indeed be catabolised by KDG aldolase. We wished to determine the kinetic parameters for both **D-KDX** and **L-KDA** for comparison with the previously recorded kinetic parameters for **D-KDG** and **D-KDGal**. Consequently, an efficient chemical synthesis of enantiopure **D-KDX** and **L-KDA** was also required to enable us to carry out kinetic studies on these sugars using KDG aldolase.

Scheme 3 *The catabolism of D-xylose **8** and L-arabinose **9** in S. solfataricus via a non-phosphorylative Entner-Doudoroff pathway*

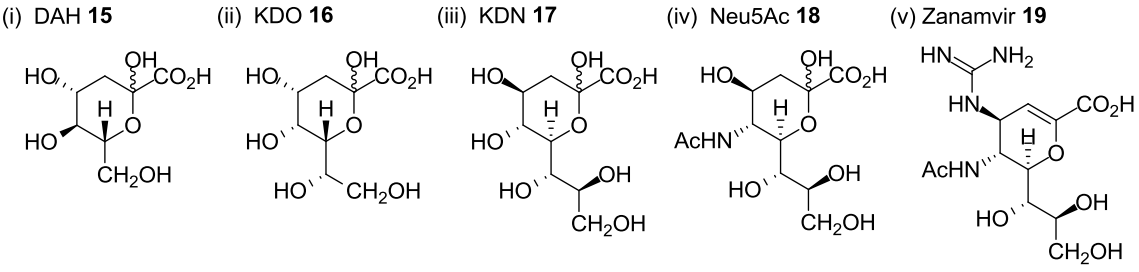


1.6 Previous Syntheses of Higher 2-Keto-3-Deoxy-Ulosonic Acids

The synthesis of the higher 2-keto-3-deoxy-ulosonic acids is an important research area as many compounds of this class have been shown to be important components of biologically important processes.[33] Thus, DAH **15** (Figure 4) is an intermediate in the

shikimate pathway, which in plants and micro-organisms is responsible for the biosynthesis of aromatic amino acids from glucose.[34-35] Its synthesis has received much attention as it is thought that selective disruption of the shikimate pathway could lead to inhibition of micro-organism growth.[36] Contrastingly, KDO **16** is an essential constituent of lipopolysaccharides on the cell surfaces of Gram-negative bacteria, where they play a role in cell-cell communication.[37] Developing novel anti-bacterial agents that target the biosynthetic pathway of KDO **16** is a promising area of research as anti-bacterial agents could be selective and efficacious, since KDO **16** is not found as a constituent on the surface of human cells. However, in bacteria KDO **16** is a critical building block for assembly of lipopolysaccharide membranes, and so interrupting its biosynthesis would seriously undermine bacterial cell viability.[38] The C9-sugars KDN **17** and Neu5Ac **18**, commonly termed sialic acids, of which there are more than 50 naturally occurring members, are found in humans and higher animals as well as in protozoa, viruses and bacteria. Amongst diverse biological functions they are known to be very important in cellular and molecular recognition, including proliferation of cancer cells and infection by microbes.[39] Since von Itzstein and co-workers found that Zanamvir **19** is an effective inhibitor of the influenza virus neuraminidase (IC_{50} = 1nM) for the treatment for influenza A and B, many drug development programmes have focused on the development of sialic acid derivatives as potential antiviral treatments.[40]

Figure 4 *Structures of higher 2-keto-3-deoxy-ulosonic acids*

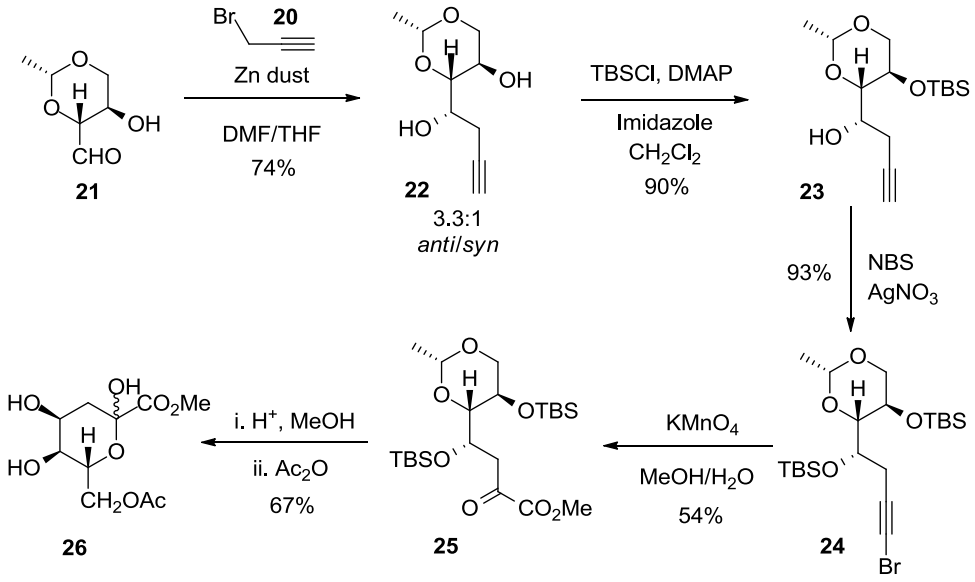


The majority of the syntheses of **15**-**19** described to date involve the addition of a suitable C2- or C3-nucleophile to a suitable sugar residue, and further functional group elaboration to a particular 2-keto-3-deoxy-ulosonic acid target. Herein, some of the more important synthetic strategies that have been used to prepare this important class of sugar natural product will be described.

1.6.1 Propargylic Nucleophilic Addition Methodology

Of the various nucleophilic reactions employed in the synthesis of 2-keto-3-deoxy-ulosonic acids, propargylic anions have received much attention. Initially Wu and co-workers developed a two step methodology for the selective oxidation of terminal alkynes to 2-keto-esters,[41] which along with diastereoselective propargylation allowed them to synthesise the protected methyl ester of the C4-epimer of DAH **26** (Scheme 4).[42] They found that reaction of propargyl bromide **20** in the presence of zinc dust with aldehyde **21** (derived from glucose) provided the C7-terminal alkyne **22** in an anti/syn ration of 3.3:1. The anti isomer was purified and then TBS protected to afford **23**. Oxidation that involved treatment of **23** with NBS in the presence of $AgNO_3$ to form bromoalkyne **24**, followed by $KMnO_4$ oxidation in a methanol/water solvent system to give **25** in 43% yield. **25** was then deprotected under acidic conditions and acetylated to give the target compound **26** in an overall yield of 24% from **20**.

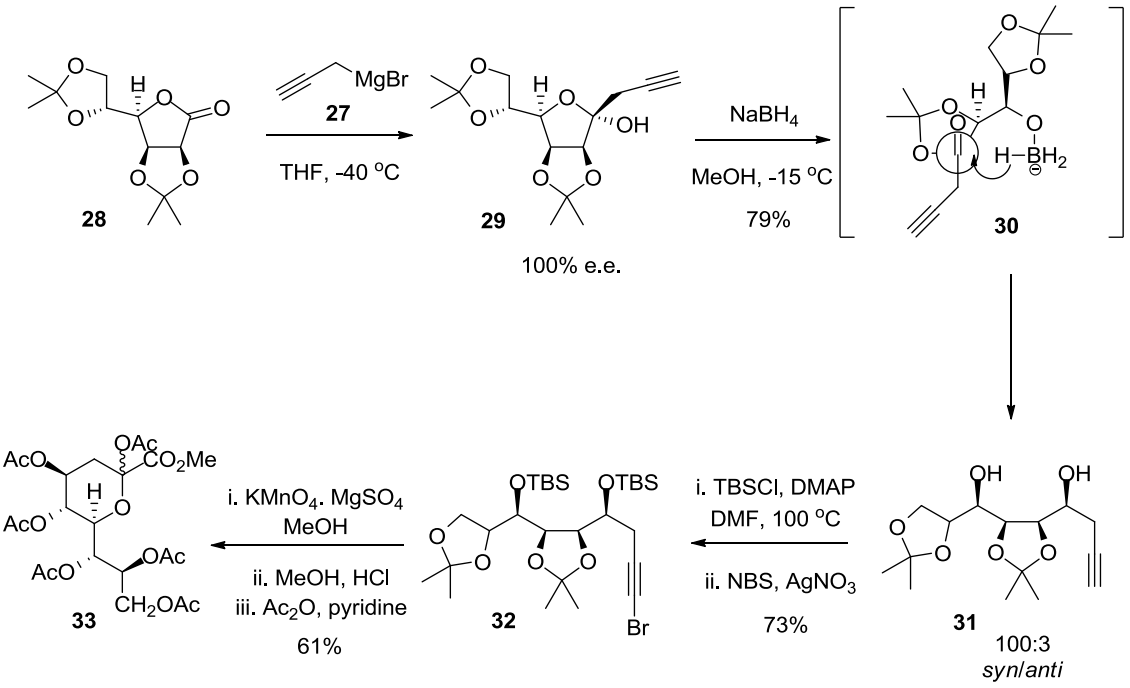
Scheme 4 *Wu synthesis of DAH C4-epimer **26***



Wu and co-workers went on to employ this methodology for the synthesis of KDO **16** and Neuc5Ac **19**,[43-44] whilst Crich and Navuluri have shown that the protected methyl ester of KDN **17** can be prepared using a modification of this protocol (Scheme 5).[45] Thus, they found that reacting propargyl magnesium bromide **27** with lactone **28** gave a single product isomer **29**, which was presumed to be due to chelation of the organometallic reagent to the lactone ether groups. Reduction of **29** proceeded with excellent *syn* selectivity when $NaBH_4$ was used at -15 °C in methanol to give **31** (*syn*/*anti* 100:3), which

the authors accredited to an alkoxyborohydride intermediate **30** being generated in the course of lactol ring opening, that resulted in hydride ion being delivered intramolecularly to give the *syn* diastereomer. A number of methods were then investigated for the terminal alkyne oxidation, with the two step NBS/$KMnO_4$ procedure of Wu, followed by deprotection and peracetylation, giving the best results for large scale synthesis of the protected methyl ester of KDN **33** in 35% overall yield.

Scheme 5 *Crich and Navuluri synthesis of protected KDN methyl ester **33***

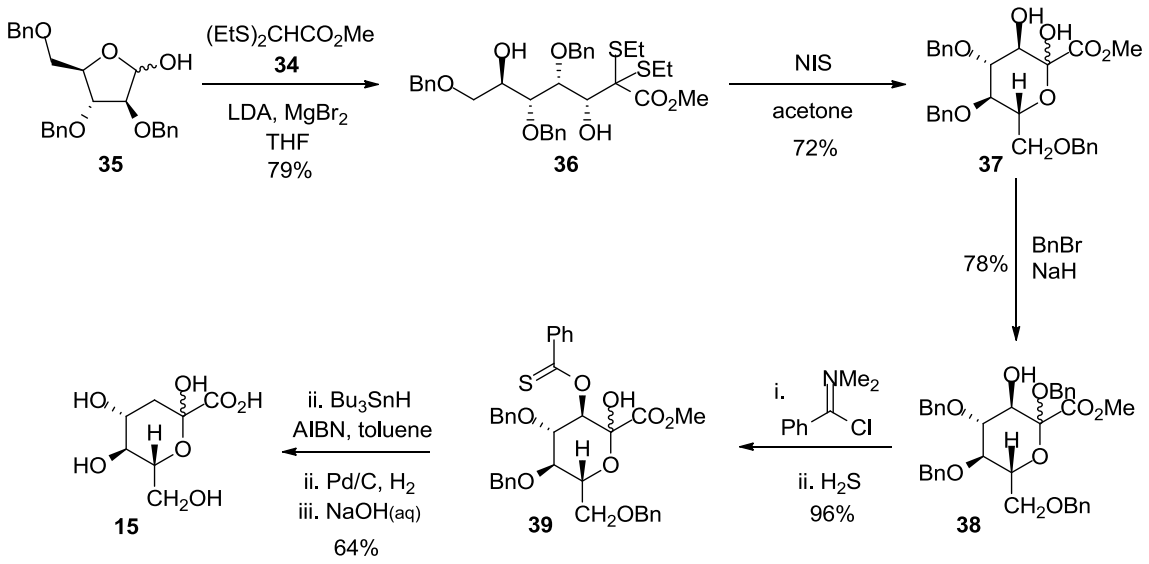


1.6.2 Dithiane Nucleophilic Addition Methodology

Schmidt and co-workers have shown that dithiane esters of the general type **34** can act as good nucleophiles for the synthesis of DAH **15** and KDO **16**.[46-47] For the synthesis of DAH **15**, addition of lithiated dithio ester **34** in the presence of magnesium bromide to benzyl protected D-arabinose **35** afforded the *syn* addition product **36** (Scheme 6). This dithiane was then treated with *N*-iodosuccinimide to cleave the dithiane, with the resultant lactol **37** being benzyl protected selectively at C(2) using benzyl bromide with sodium hydride as base. Free radical deoxygenation of the C(3) hydroxyl group of **38** using Barton's procedure was achieved by converting the C(3) hydroxyl into its 3-*O*-thiobenzoyl derivative that proceeded in quantitative yield, followed by treatment with tributyltin hydride and AIBN to yield **39**.[48] Subsequent hydrogenolysis of the benzyl groups gave

KDN **15** in an overall yield of 27% over seven steps. More recently Yamasaki and co-workers have shown that dithiane **34** can also be reacted with a mannofuranose-5,6-cyclic sulphate to stereoselectively introduce the C2-fragment.[49] They then used a similar strategy to Schmidt to unmask the α-keto acid moiety and deprotect the sugar to afford KDO **16** in 26% overall yield over seven steps.

Scheme 6 *Shmidt synthesis of DAH **15** utilising a dithiane ester*

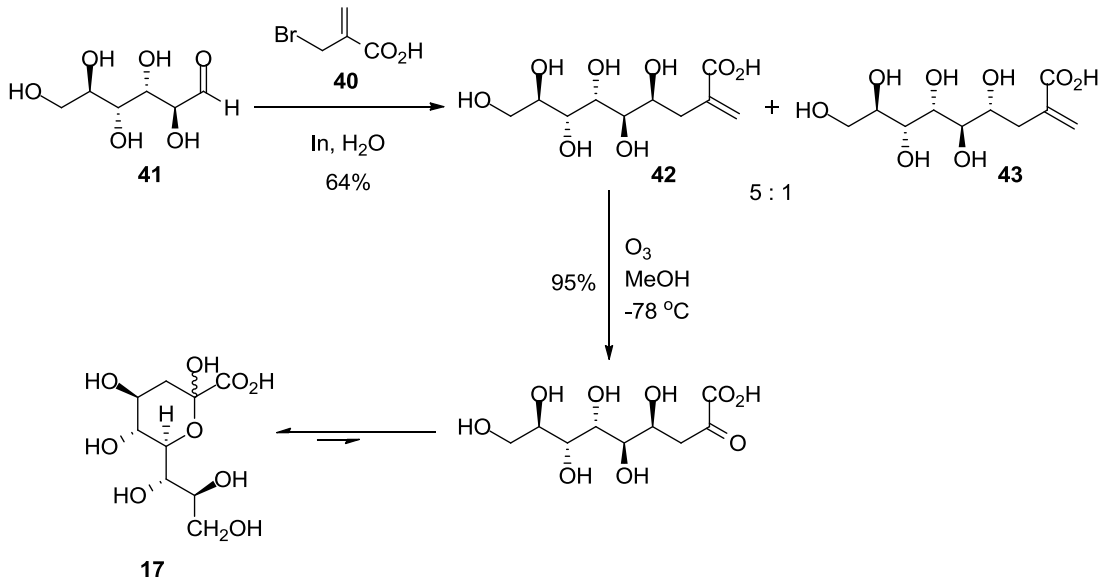


1.6.3 Acrylate Nucleophilic Addition Methodology

Ethyl α-(bromomethyl)-acrylate **40** is a noteworthy example of a C3-synthon that has been demonstrated to undergo diastereoselective tin and indium mediated addition to aldoses, allowing rapid access to 2-keto-3-deoxy-ulosonic acids.[50-52] Thus Chan and Li prepared KDN **17** in just two steps from D-Mannose **41** in an impressive 61% yield (Scheme 7). Initial coupling of **40** and **41** in the presence of indium in water proceeded to afford a mixture of diastereomers **42** and **43** with good levels of diastereoselectivity (*syn*/*anti* 5:1). The choice of water as the reaction medium was key to the success of the synthesis as it allowed an unprotected sugar to be used as a substrate, obviating the requirement for protection and deprotection of the alcohol functionalities. Recrystallization from methanol/ethyl acetate provided the pure *syn* diastereomer **42** in an excellent 64% yield. The α-keto acid functionality was then revealed by ozonolysis of the alkene group to afford **17** in 95% yield from **41**. Whitesides has extended this methodology to other 2-keto-3-deoxy-ulosonic acids,[52] in particular reporting that C(2) acetylated nitrogen aldoses also

undergo diastereoselective coupling with **40**, which provided direct access to derivatives of Neu5Ac **18** over just three steps from suitably functionalised *N*-aldoses.

Scheme 7 *Chan and Li synthesis of KDN **17***



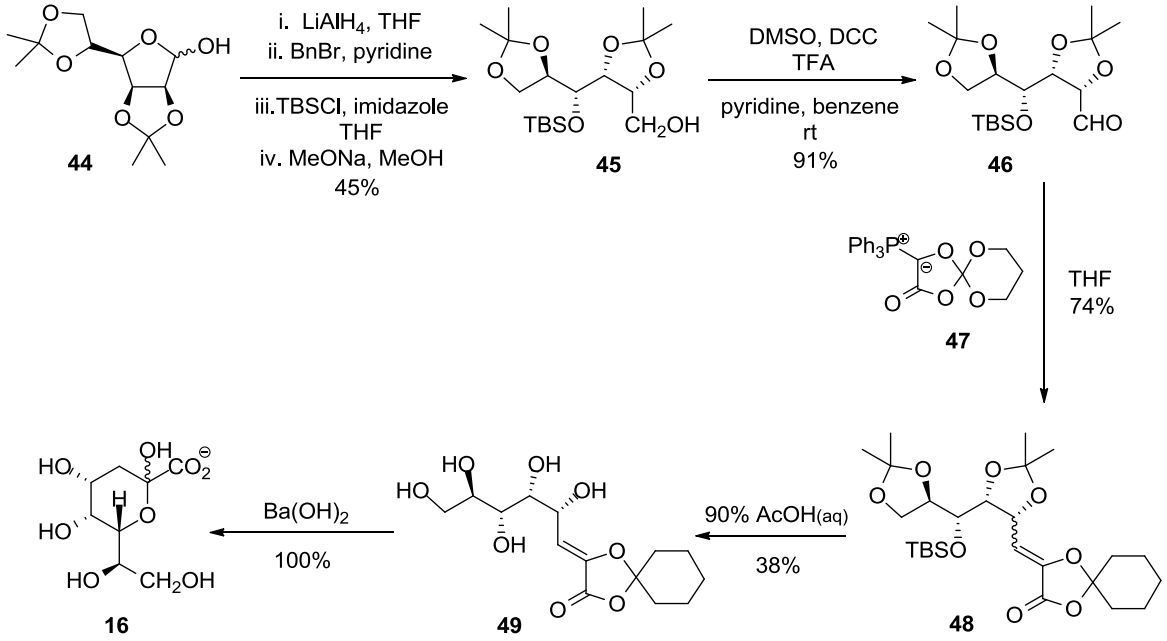
1.6.4 Horner-Wadsworth-Emmons Methodology

Ramage and co-workers showed that the use of a Horner-Wadsworth-Emmons (HWE) reaction was a powerful strategy for the introduction of a masked α-keto acid group, demonstrating their methodology for the synthesis of DAH **15** and KDO **16** (Scheme 8).[53] Thus **45**, which had been prepared from D-mannose by a series protection, reduction, deprotection and oxidation steps was oxidised to aldehyde **46**. Reaction of this aldehyde with Wittig reagent **47** gave the adduct **48** as a mixture of *cis*- and *trans*-geometrical isomers that could be deprotected by sequential acid and base treatment to give KDO **16** in an overall 12% yield.

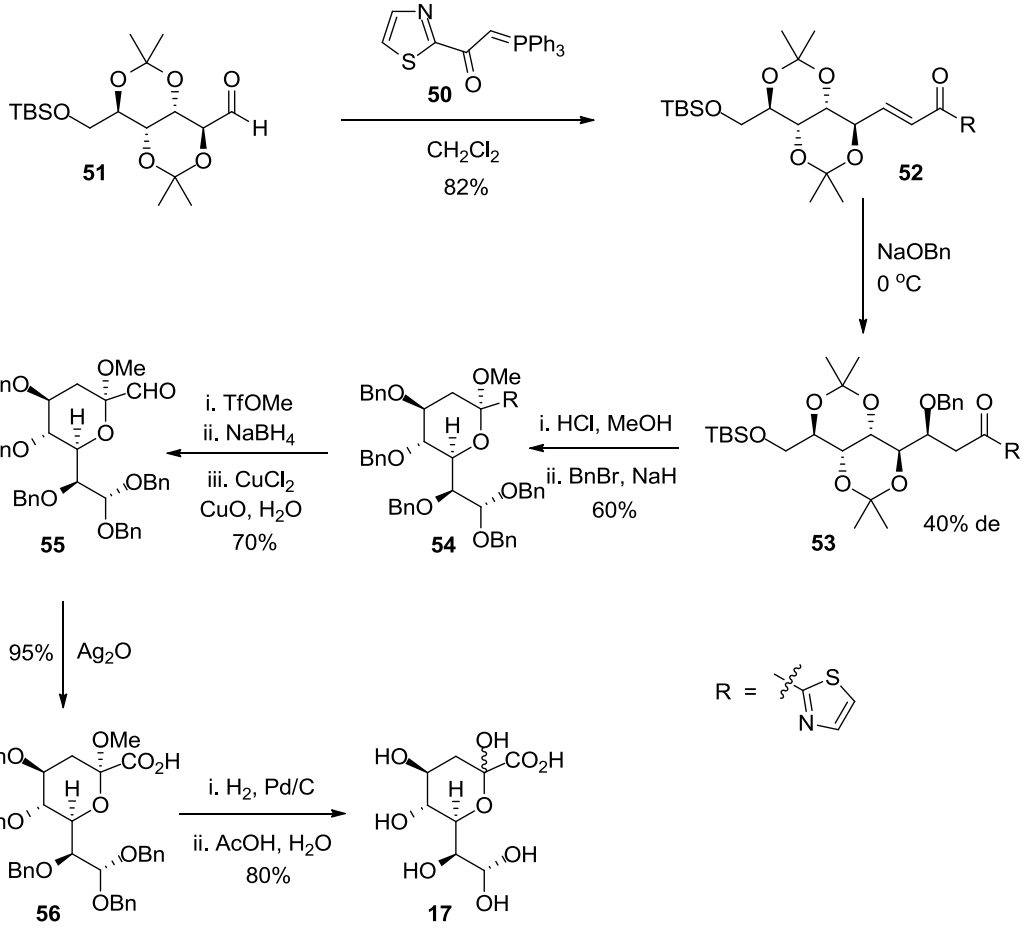
Later Dondoni and co-workers demonstrated that an alternative ylid **50** could be successfully reacted with protected sugars for the synthesis of DAH **15** and KDN **16**.[54] An initial Wittig reaction was used to couple a protected derivative **51** of D-mannose with ylid **50** to give **52** in 82% yield (Scheme 9). This was then subjected to an *oxa*-Michael reaction that proceeded under thermodynamic control with mediocre stereoselectivity (syn/anti 70:30), however the major *syn*-addition product **53** could be isolated by flash chromatography in 54% yield. Later the acyl thiazole moiety was converted into an aldehyde group and then oxidised with Ag_2O to give the required α-keto acid functionality,

further functional group isomerisations eventually afforded KDN **17** in an overall yield of 14% from **51**.

Scheme 8 *Ramage and co-workers HWE synthesis of KDO **16***



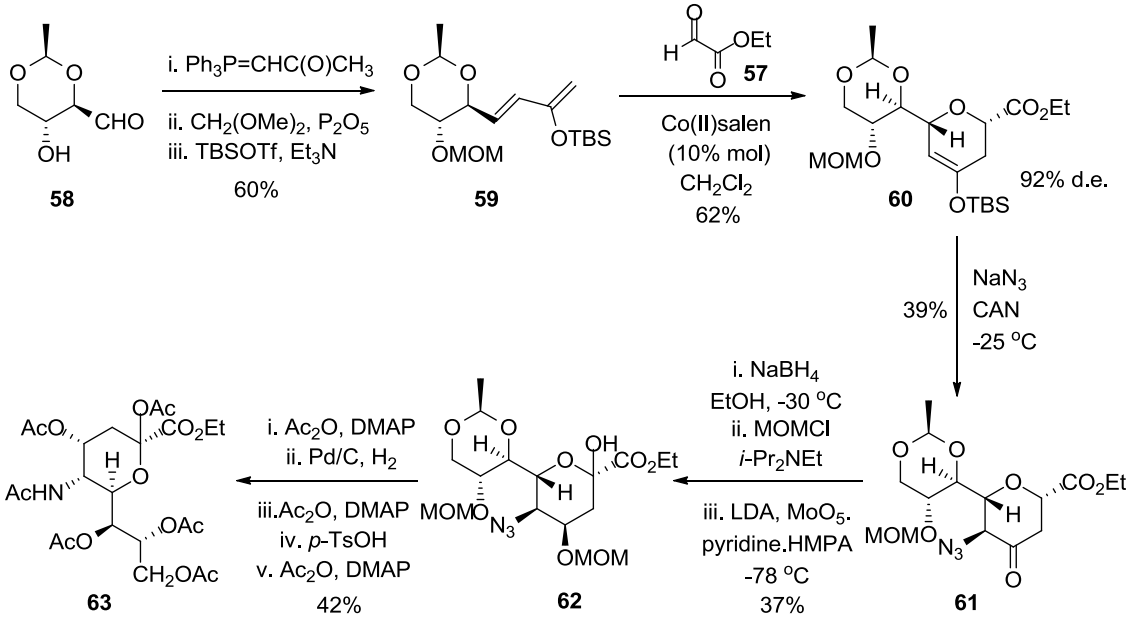
Scheme 9 *Dondoni and co-workers HWE synthesis of KDN **17***



1.6.5 Pericyclic Approaches for the Synthesis of 2-Keto-3-Deoxy-Ulosonic Acids

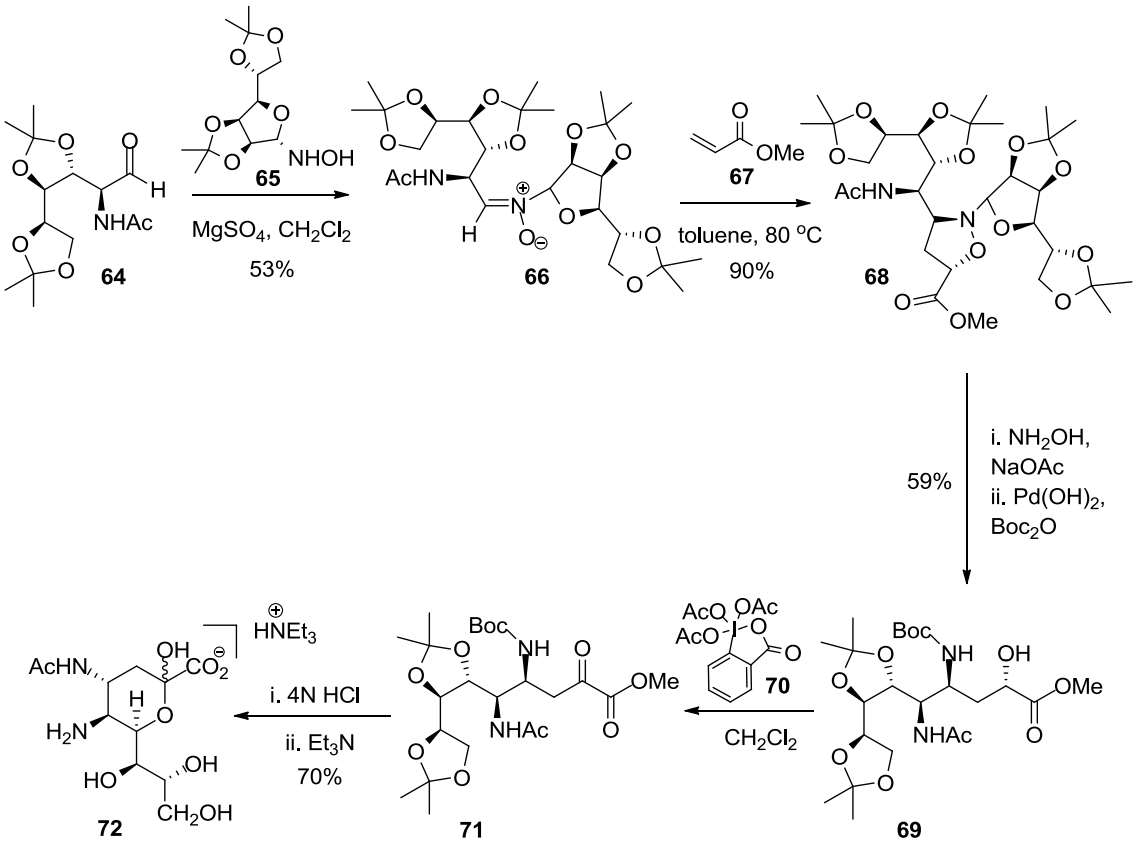
Queneau and co-workers first showed that the hetero Diels-Alder reaction could be applied for the synthesis of 2-keto-3-deoxy-ulosonic acid derivatives, although they found that the reaction proceeded with poor stereoselectivity.[55] Wu and co-workers then showed that better levels of stereoselectivity could be achieved when a Lewis acid was added to the hetero Diels-Alder reaction of suitable dienes with ethyl glyoxylate **57**, which allowed them to synthesise various derivatives of KDO **16** and DAH **17**.[56-58] More recently they showed that this methodology could be applied to the total synthesis of the peracetylated ethyl ester of Neu5Ac **18** (Scheme 10).[59] Thus, Wittig reaction of aldehyde **58** with a phosphonium anion gave an enone that was protected as its MOM ether and treated with TBSOTf and Et_3N to give silyl-enol ether **59** in 60% yield over the three steps. The hetero Diels-Alder reaction between **59** and ethyl glyoxylate **57** catalyzed by (salen)cobalt(II) complex afforded **60** in 62% isolated yield with a d.e. of 92%. After trialing a number of methods for the introduction of electrophilic nitrogen functionality at C(5) treatment with sodium azide (3 equiv.) in the presence of CAN (2.5 equiv.) at reduced temperature gave 62% yield of the desired azide isomer **61**. This was stereoselectively reduced using $NaBH_4$ and the resultant alcohol protected as its MOM ether. Oxidation at C(2) to create the crucial α-keto acid functionality was achieved by treating the MOM ether with MoO_5.Py.HMPA to afford **62**, and then a series of deprotection/reduction/protection steps to furnish the desired Neu5Ac derivative **63** in an overall yield of 3% in eleven steps.

Scheme 10 *Hetero Diels-Alder synthesis of the ethyl ester of protected sialic acid **63***



Yao and co-workers went on to demonstrate that a toluene solution of nitrone **66** undergoes a highly stereoselective 1,3-dipolar cycloaddition reaction when heated in the presence of methyl acrylate **67** (Scheme 11).[60] Initially aldehyde **64** was reacted with a sugar-derived hydroxylamine **65** in the presence of $MgSO_4$ to furnish nitone **66** bearing Vasella's chiral auxiliary. The 1,3-dipolar cycloaddition reaction of nitrone **66** with methyl acrylate **67** then afforded isoxazolidine **68** as the major product in 90% yield. Removal of the chiral auxiliary followed by reductive cleavage of the N-O bond of the isoxazolidine revealed α-hydroxy-γ-amino carboxylate **69** with the desired stereochemistry. Dess-Martin periodinane **70** then successively oxidised the α-alcohol group to α-keto acid **71**, which was deprotected to afford 2-keto-3-deoxy-ulosonic acid **72** in an overall yield of 20% in eight steps.

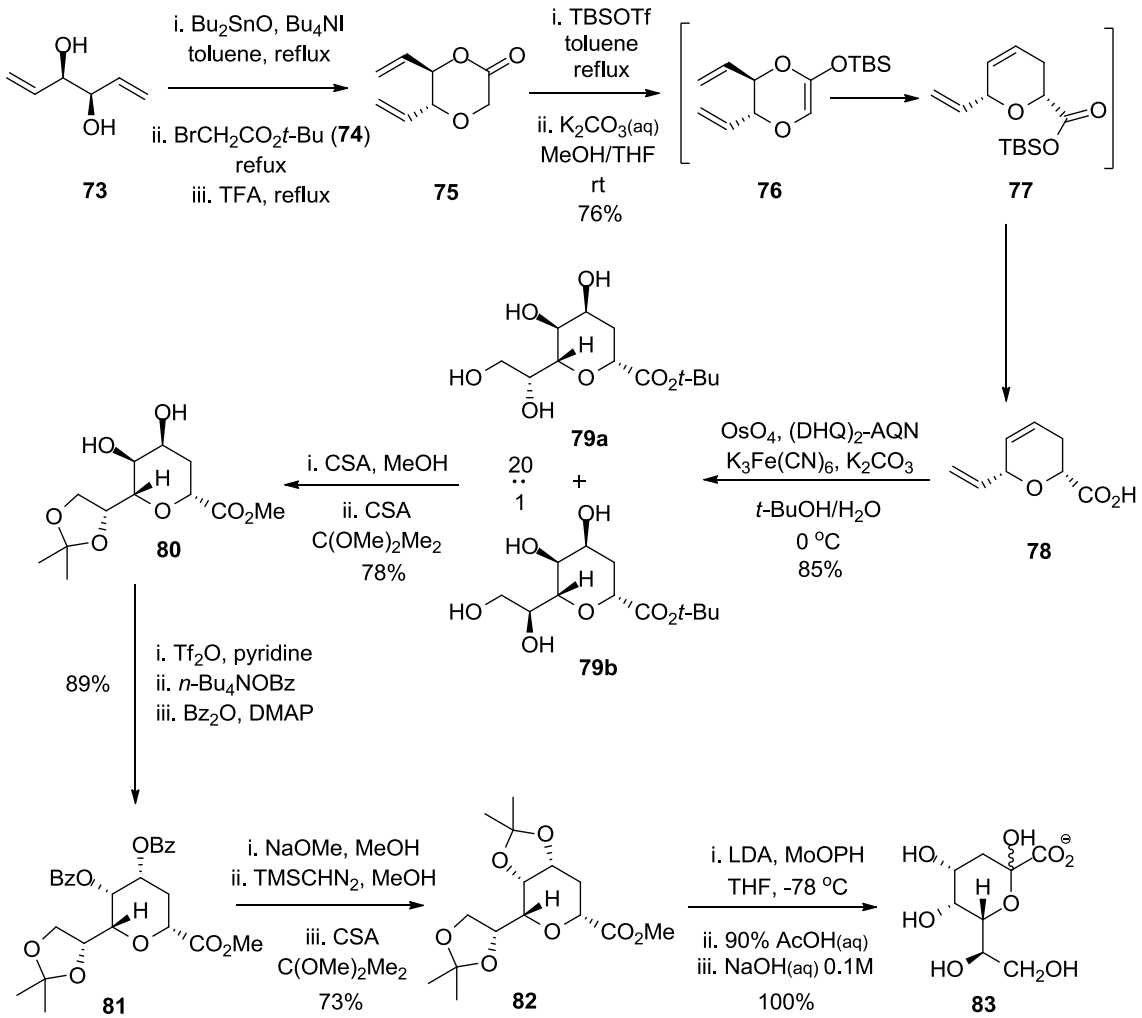
Scheme 11 *1,3-Dipolar cycloaddition synthesis of sialic acid derivative **72***



Burke and co-workers have shown that the Ireland-Claisen [3,3]-sigmatropic rearrangement can also be employed for the synthesis of 2-keto-3-deoxy-ulosonic acids (Scheme 12).[61] Initially diene **73** was synthesised over four steps from D-mannitol.

Alkylation of the stannylene acetal of **73** by *tert*-butyl bromoacetate **74**, that proceeded with concomitant cyclisation provided the desymmetrized dioxane **75**. Conversion of **75** to the silyl-ketene acetal **76**, followed by Ireland-Claisen [3,3]-sigmatropic rearrangement and hydrolysis of the intermediate silyl-ester allowed for the stereoselective synthesis of **78** in 76% yield. Double asymmetric Sharpless dihydroxylation was then trialed using a range of chiral ligands, with the endocyclic double bond being dihydroxylated from the undesired face in all cases, which the authors attributed to the C(6) substituent overriding the influence of the chiral ligands. Therefore the configuration of the diol fragment was inverted and then oxidation at C(2), using the Wu methodology (*vide supra*), and deprotection afforded KDO **16** in an overall yield of 17% from D-mannitol.

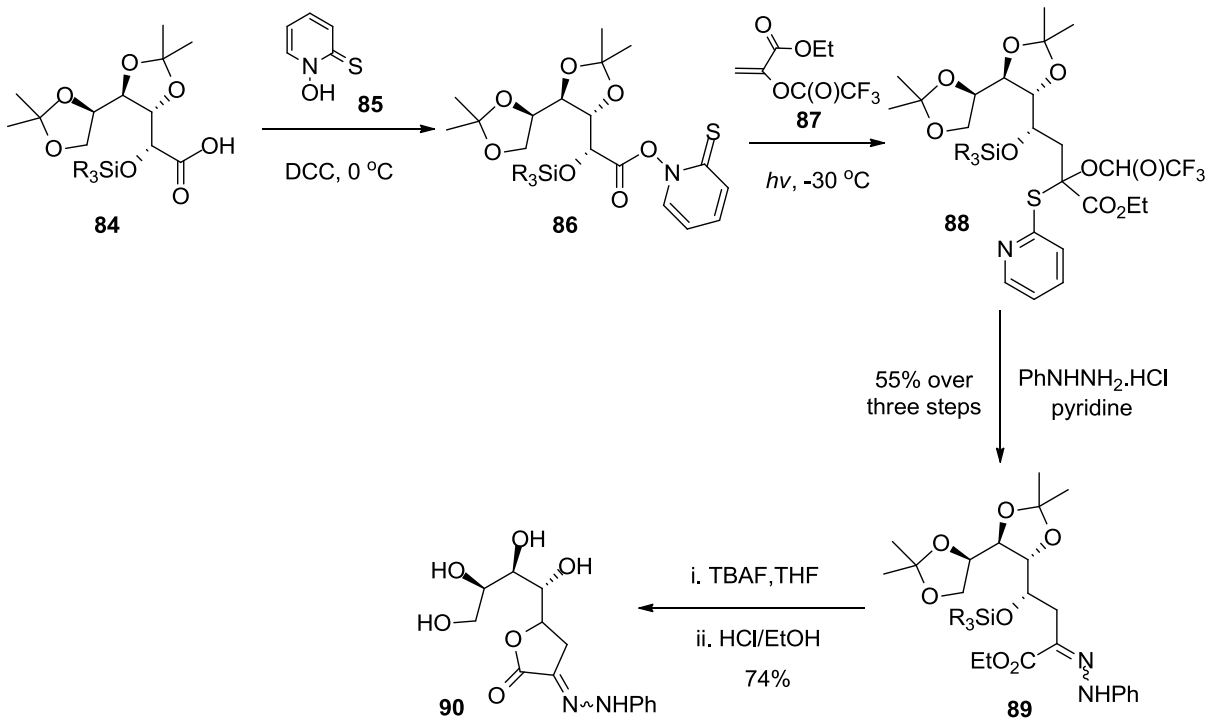
Scheme 12 *Burke Ireland-Claisen [3,3]-sigmatropic rearrangement synthesis of KDO **16***



1.6.6 Radical Methodology

Early work on allylic radicals and Barton ester radicals showed that single electron reactions could be used for the synthesis of α-keto acids under mild reaction conditions.[62-67] Barton's approach is exemplified by his synthesis of KDO derivative **90** from a readily accessible protected aldonic acid **84** (Scheme 13). Coupling of *N*-hydroxy-2-thiopyridone **85** with the acid fragment of **84**, gave Barton ester **86** that underwent radical reaction with ethyl α-(-trifluoro-acetoxy)acrylate **87** when irradiated with UV light. Intermediate **88** was not isolated, but reacted *in situ* with phenylhydrazine in pyridine to give the phenylhydrazone derivative **89**. Sequential treatment of **89** with TBAF, followed by hydrochloric acid in ethanol afforded the hydrazone derivative of KDO **90** in an overall yield of 41% over five steps.

Scheme 13 *Barton radical decarboxylation reaction for the synthesis of KDO derivative **90***

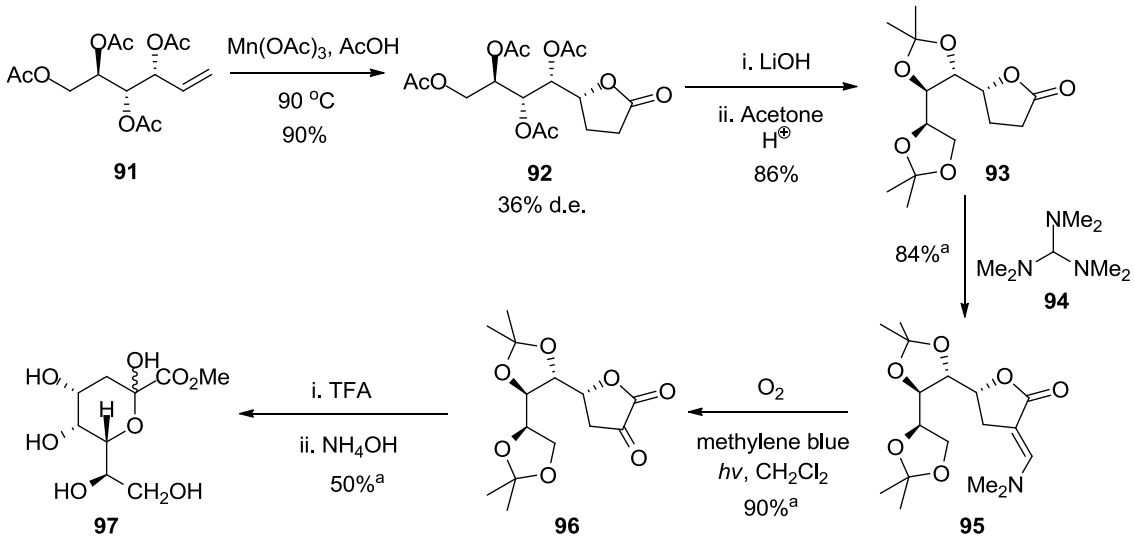


More recently, in the course of their studies into transition metal mediated radical reactions, Linker and co-workers have developed complimentary oxidative and reductive radical reactions for the formal synthesis of the C(7) epimers of KDO **96** and **102** (Scheme 14).[68] They discovered that manganese (III) acetate promotes the oxidative radical coupling of acetic acid with acyclic alkene **91** in an excellent yield of 90%, but with a mediocre stereoselectivity of 36% d.e. Attempts to optimise the reaction conditions did not improve the diastereoselectivity, although the product diastereomers could be

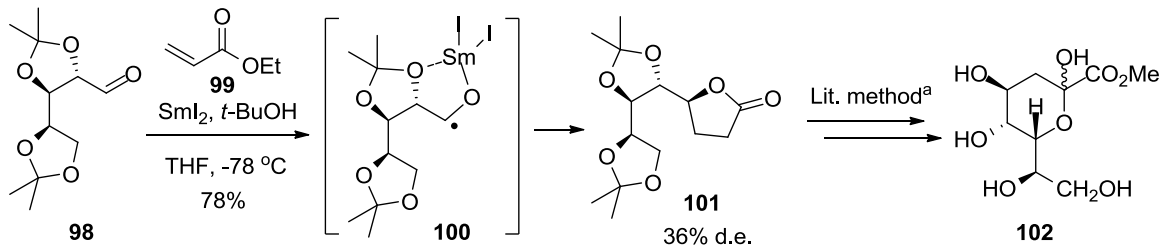
separated by flash chromatography, allowing **92** to be isolated and converted to lactone **93**, which had been previously advanced to *manno*-KDO **97** by condensation with tris(dimethylamino)-methane **94**, followed by treatment with singlet oxygen generated *in situ* by bubbling oxygen through a solution of **94** in the presence of a catalytic quantity of methylene blue whilst irradiating the reaction mixture with a Argaphoto-B lamp. Acid catalysed hydrolysis of the resultant 2-keto-lactone **96** afforded the target 2-keto-3-deoxy-ulosonic acid **97**.[69] Alternatively, they demonstrated that a carbohydrate fragment could act as the radical precursor in the presence of a suitable metal. Thus protected sugar **98** was reacted with ethyl acrylate **99** and samarium (II) iodide in the presence of various additives. The best coupling reactions were attained when *tert*-butanol was used as an additive at -78 °C, affording the key intermediate **101** in 78% yield, albeit with mediocre stereoselectivity of 36% d.e. The reversal of diastereoselectivity compared to the manganese (III) acetate mediated radical reaction was rationalized by invoking transition state **100** where the *O*-isopropylidene group shields one face of the samarium complex, causing the acrylate to preferentially approach the radical from the *Si* face.

Scheme 14 *Formal synthesis of KDO C(7) epimers (i)* ***97*** *and (ii)* ***102*** *via radical coupling procedure*

(i)



(ii)

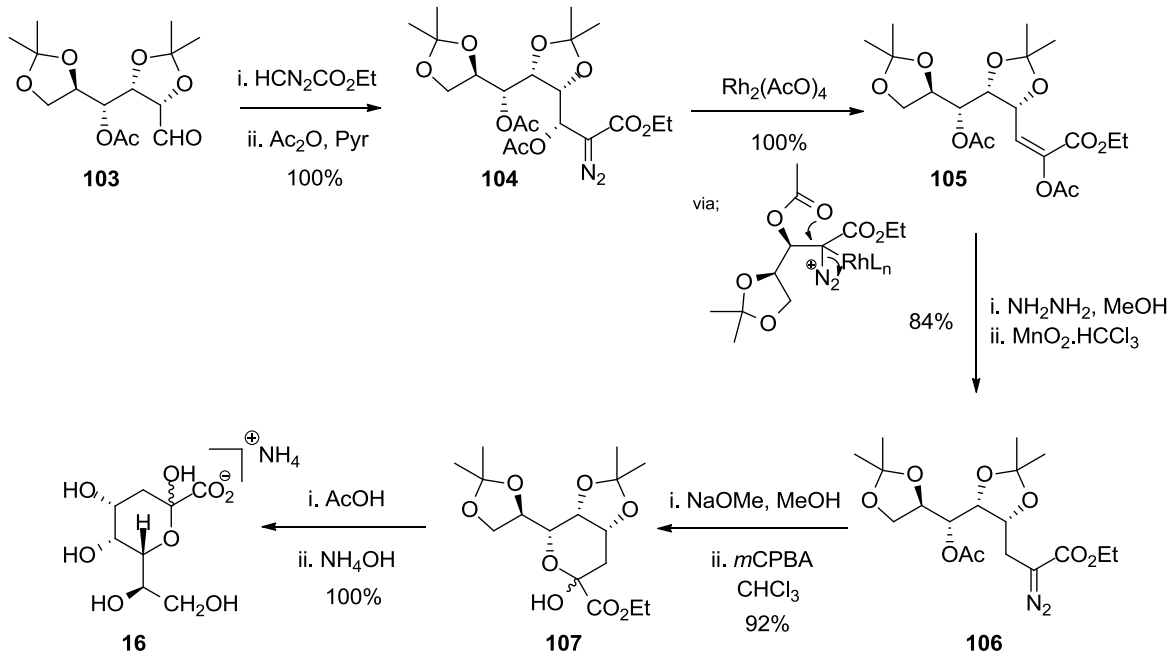


(a. completed with literature methodology for KDO ***16*** *synthesis from intermediate* ***101****[69])*

1.6.7 Rhodium-Mediated Carbenoid Rearrangement Methology

López-Herrera and co-workers have demonstrated the efficacy of rhodium mediated carbene chemistry for the synthesis of KDO **16**, KDN **17** and other derivatives.[36,70] Thus, initial reaction of ethyl diazoacetate with protected sugar **103**, and subsequent treatment with acetic anhydride in pyridine provided **104**, which underwent facile rhodium mediated rearrangement of the acetyl substituent that has been proposed to proceed *via* carbonyl oxygen nucleophilic attack on the intermediate rhodium species, which affords **105** in excellent yield (Scheme 15). Transformation of enol ester **105** to the corresponding 2-hydrazone derivative, followed by oxidation with MnO_2 gave the diazo compound **106**. Attempted deprotection of the carbenoid group and insertion into an alcohol bond using UV light or silver oxide proved unsuccessful, however when acetyl deprotection with sodium methoxide was followed by oxidation with by *m*CPBA the desired product **107** was isolated as the sole product, which after acetonide deprotection gave KDO **16** in a reported yield of 77% over nine steps.

Scheme 15 *Synthesis of KDO **16** via rhodium mediated rearrangement of a carbenoid species*

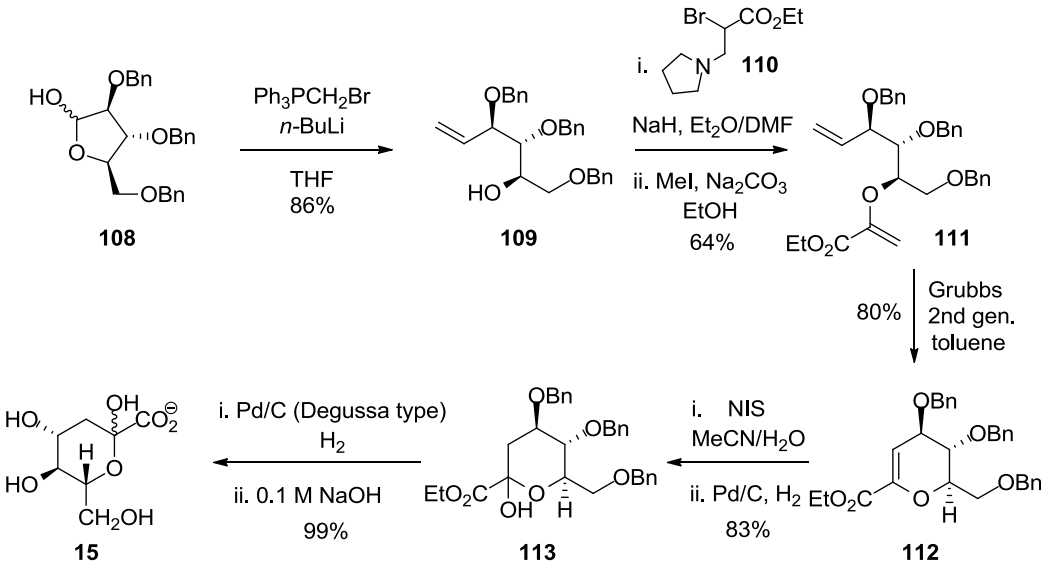


1.6.8 Metathesis Methodogy

Ring closing metathesis has been applied for the synthesis of key intermediate ring systems for the synthesis of KDN **17**, Neuc5Ac **18** and other 2-keto-3-deoxy-ulosonic acid derivatives.[71-73] Most recently Rutjes and co-workers have reported the synthesis of DAH

15 starting from protected sugar **108**, involving ring closing metathesis of a highly substituted α-alkoxyacrylate **111**, with subsequent functionalization *via* an iodohydrin elimination (Scheme 16). Alcohol **109** was prepared from the protected sugar **108** using standard Wittig methodology. Generation of α-alkoxyacrylate **111** involved reaction **109** with α-bromoester **110**, followed by *N*-methylation and base catalysed elimination. **111** was then treated with Grubbs 2nd generation catalyst to give enol-ether **112** in good yield. The synthesis was completed by iodohydroxylation of the enol ether bond and subsequent reduction of the iodo group to afford **113**, followed by hydrogenolysis of the benzyl groups and ester hydrolysis to afford DAH **15** in 39% overall yield over eight steps.

Scheme 16 *Rutjes total synthesis of DAH **15***



1.6.9 Organocatalytic Methodology

Improving on previous work using masked pyruvic acid equivalents in organocatalytic systems,[74-75] Enders and co-workers have recently reported a highly enantioselective proline catalysed synthesis of 2-keto-3-deoxy-ulosonic acid precursors.[76] Trialing a range of enantiomerically pure pyrrolidine analogues for the reaction of 2-methyl propanal **114a** with pyruvic acid dimethyl acetal **115** showed that (*S*)-proline **116** gave the best enantioselectivities (73% e.e.). The temperature, solvent and reaction time were optimised for the (*S*)-proline **116** catalysed reaction and the methodology was trialed with a number of aldehydes (Table 3). For the higher 2-keto-3-deoxy-ulosonic acid precursors excellent levels of stereoselectivity were recorded (≥99% e.e., 90-92% d.e.), although this success was tempered by long reaction times and low yields due to Mannich-elimination

and self-condensation of acetal **115**. The 2-keto acetal products **117a-e** are potential precursors to 2-keto-3-deoxy-ulosonic acids, but the authors did not manage to hydrolyse and oxidise the acetals to the corresponging 2-keto-ulosonic acids.

Scheme 17 *(S)-proline catalysed reaction of pyruvic acid dimethyl acetal with a range of aldehydes*

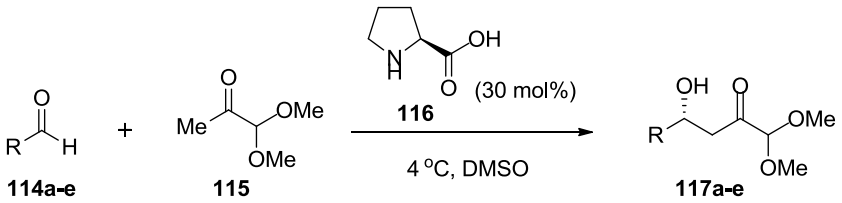
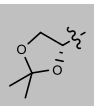
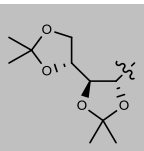


Table 3 *(S)-proline **116** catalysed reaction of pyruvic acid dimethyl acetal **115** with aldehydes **114a**-**e***

Adehyde	R	Time	Yield	% de	% e.e.
117a	*i*-Pr	8	48	-	93
117b	*c*-Hex	10	37	-	85
117c		5	38	91	≥99
117d		7	31	90	≥99
117e		9	35	92	≥99

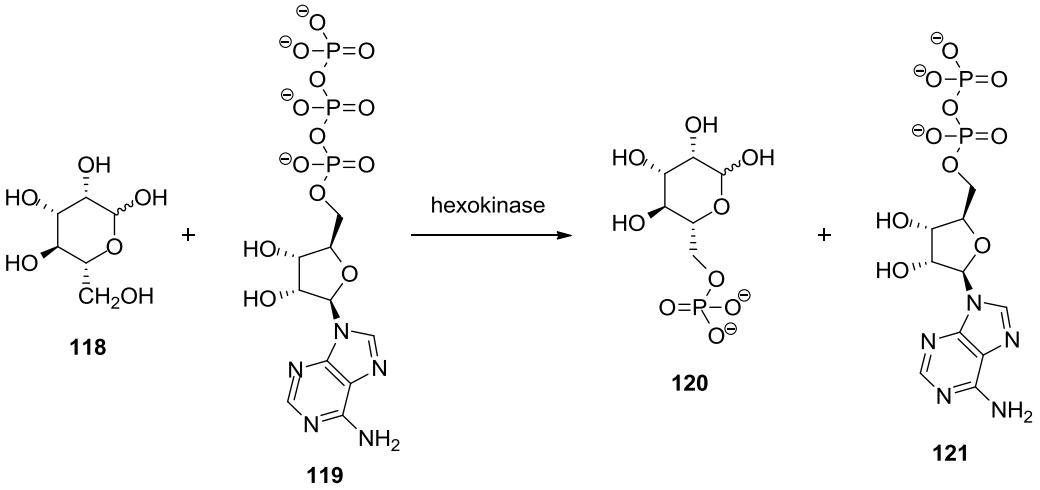
1.6.10 Enzymatic Methodology

The biosynthesis of higher 2-keto-3-deoxy-ulosonic acids normally involves the tandem action of three or more enzymes. This is exemplified by the biosynthesis of KDN **17**: (i) kinase mediated phosphorylation of a sugar precursor **118**; (ii) an aldol type reaction with pyruvate **7** (or pyruvate equivalent) mediated by an aldolase; (iii) removal of the phosphate group mediated by a phosphatase (Scheme 18).[77-79]

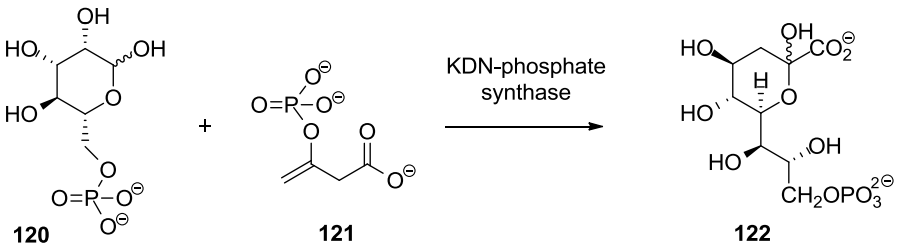
A number of groups have used the enzymes employed for the synthesis of KDO **16** and Neu5Ac **18** for the preparation of 2-keto-3-deoxy-ulosonic acid derivatives.[80-83] Wong

Scheme 18 *Biosynthesis of KDN* ***17***[79]

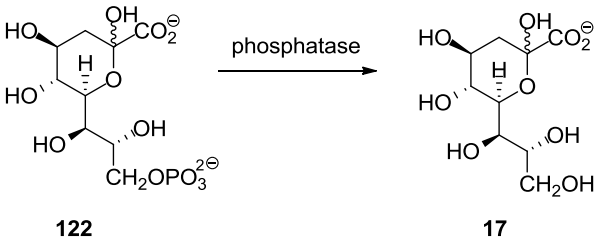
(i) *Phosphorylation*



(ii) *Aldol reaction with pyruvate enolate equivalent*



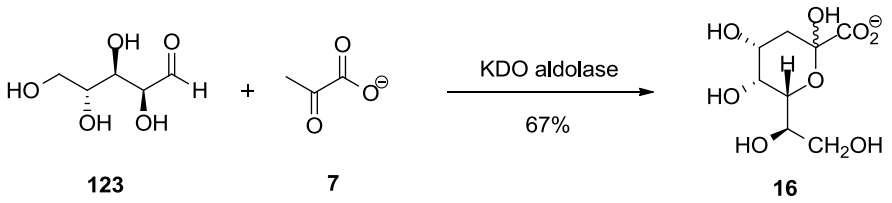
(iii) *Dephosphorylation*



and co-workers published particularly impressive results for the synthesis of KDO **16** and its analogues.[82] They first identified a Gram-negative bacterium, *Aureobacterium barkerei*, which contained high levels of KDO aldolase, and then isolated the bacterium's aldolase in a more pure form by ammonium sulphate precipitation. This enzyme catalysed reaction of D-arabinose **123** with pyruvate **7**, using an excess of pyruvate **7** to drive the equilibrium towards aldol products, affording the desired aldol product in a yield of 67% after purification by ion exchange chromatography. The substrate scope was studied and it was shown that more than 20 natural and unnatural sugars were accepted as aldose substrates. They observed that substrates with an *R*-configuration at C(3) gave

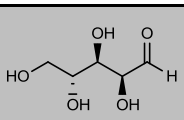
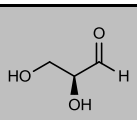
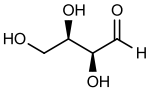
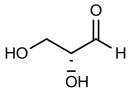
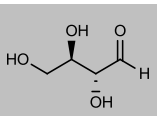
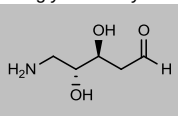
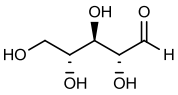
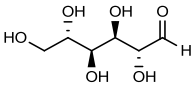
higher rates of aldol reaction (Table 4). It was also discovered that the substituent on C(2) of the sugar had little effect on stereoselectivity of the reaction, which was governed by the enzyme delivering pyruvate **7** to the *Re* face of the aldehyde.

Scheme 19 *Synthesis of KDO **16** by KDO aldolase from Aureobacterium barkerei*



123 **7** **16**

Table 4 *Substrate scope of KDO aldolase from Aureobacterium barkerei*

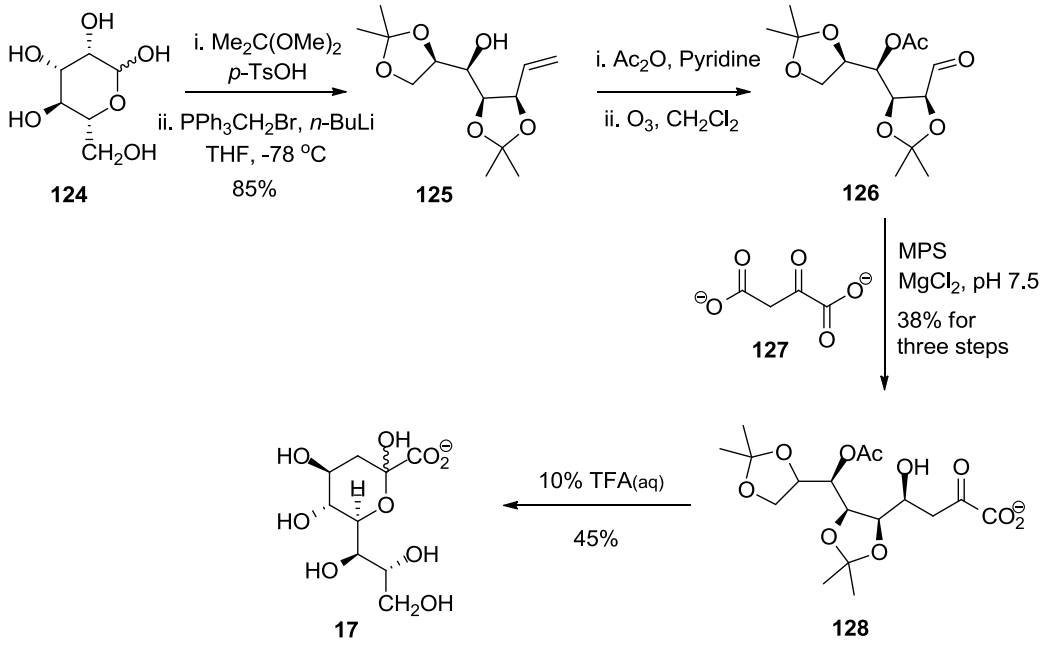
Sugar	Relative rate[a]	Sugar	Relative rate[a]
 D-arabinose	100	 L-glyceraldehyde	71
 D-threose	128	 D-glyceraldehyde	36
 **D-erythrose**	93	 5-azido-2,5-dideoxy-D-ribose	15
 **D-ribose**	72	 L-mannose	15

a. Measured at pH 7.5 with 500 mM sugar and 10 mM pyruvate

Hilvert and co-workers have recently published impressive results for the synthesis of 2-keto-3-deoxy-ulosonic acids from protected sugars and oxaloacetate **127** (generates *in situ* pyruvate enolate) catalysed by the putative Diels-Alderase macrophomate synthase (MPS),[84] which has previously been used to catalyse aldol reaction of oxaloacetate **127** with a variety of aromatic and aliphatic aldehydes.[85] Good to excellent levels of diastereoselectivity were achieved for a variety of protected sugars (from 4:1 to >19:1 dr), so the total synthesis of KDN **17** was attempted starting from protected D-mannose **124**

(Scheme 20). The aldol reaction proceeded with a good dr of 8:1, but with a mediocre yield of 39% that was attributed in part to the instability of the aldehyde starting material under aqueous conditions. Global deprotection of **128** by treatment with 10% TFA afforded KDN **17** in 14% yield over six steps.

Scheme 20 *Hilvert enzymatic synthesis of KDN **17***



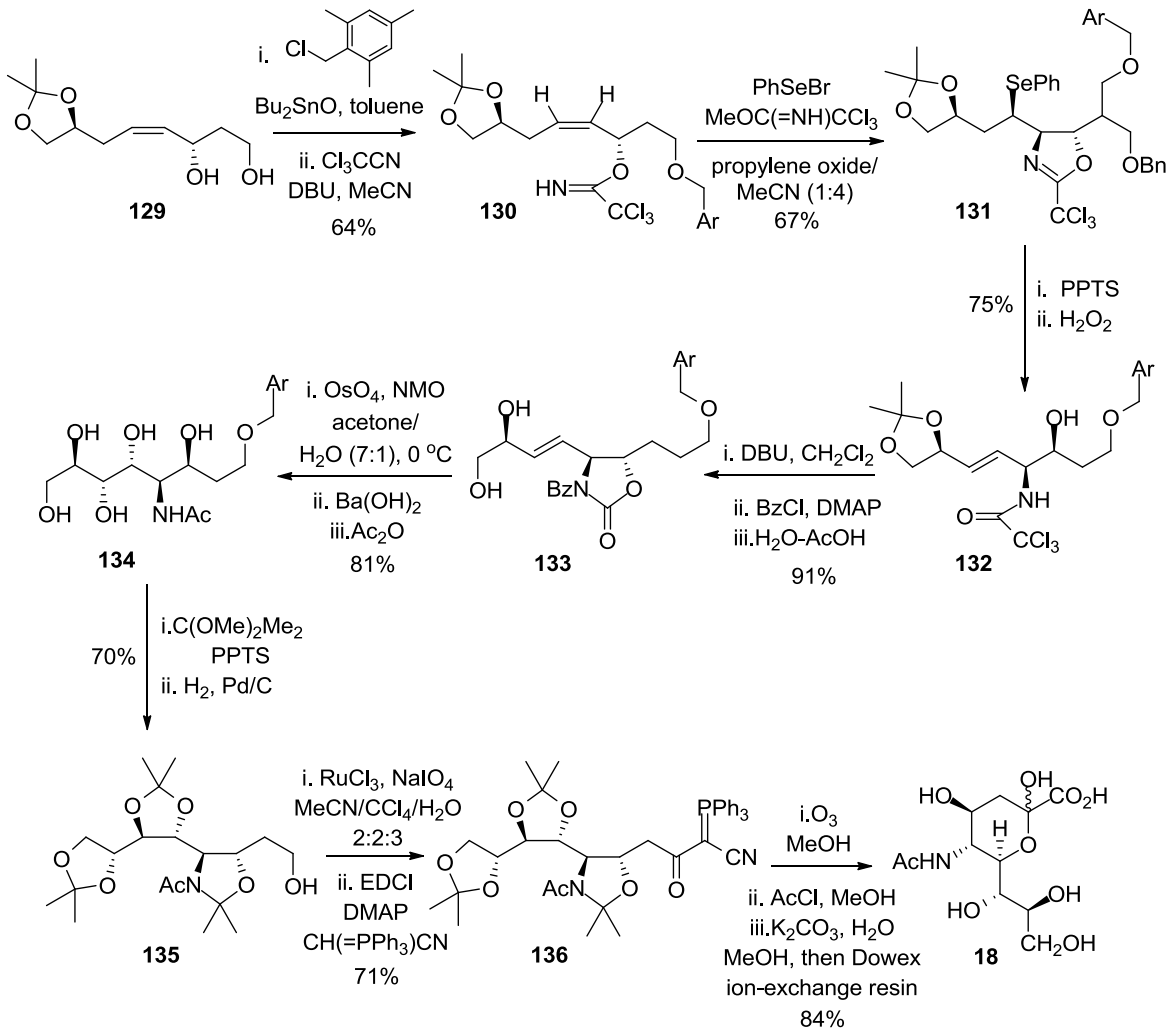
MPS - macrophomatee synthase

1.6.11 Synthesis from Non-Sugar Based Starting Materials

The synthesis of 2-keto-3-deoxy-ulosonic acids from non-sugar starting materials is a particularly difficult challenge, normally requiring a great number of synthetic steps, such as Johnson and co-workers' synthesis of DAH **15** in 23 steps from cycloheptatriene in 8% yield.[86] However, a number of groups have chosen this approach to showcase novel methodology and/or ingenious synthetic strategies.[87-91] Kang and co-workers have reported the highly diastereoselective synthesis of Neu5Ac **18** *via* intramolecular phenylselenoamidation and asymmetric dihydroxylation (Scheme 21).[91] Initially primary alcohol **129** was regioselectively benzylated and the required amino group introduced by reacting the unprotected allylic alcohol with trichloroacetonitrile in the presence of DBU to furnish **130**. Exposing **130** to benzeneselenyl bromide in the presence of an acid scavenger and dehydrating agent, propylene oxide and methyl trichloroacetimidate respectively, provided oxazoline **131** that could be partially hydrolysed in the presence of

pyridinium *para*-toluenesulfonate, and have the benzeneselenyl group oxidatively eliminated to afford *trans*-alkene **133**. After optimisation, it was found advantageous to incorporate the amine and hydroxyl groups into the oxazolidin-2-one ring of **135** that underwent highly stereoselective dihydroxylation reactions. Thus, **133** was dihydroxylated in high d.e., deprotected and the amino group re-acetylated to furnish pentaol **134** with a dr of 18:1. All the required chiral functional groups had been introduced, so the terminal benzyloxy functionality was transformed into an α-keto acid following Wasserman's protocol, followed by acetonide deprotection to afford Neu5Ac **18** in an overall yield of 10%.

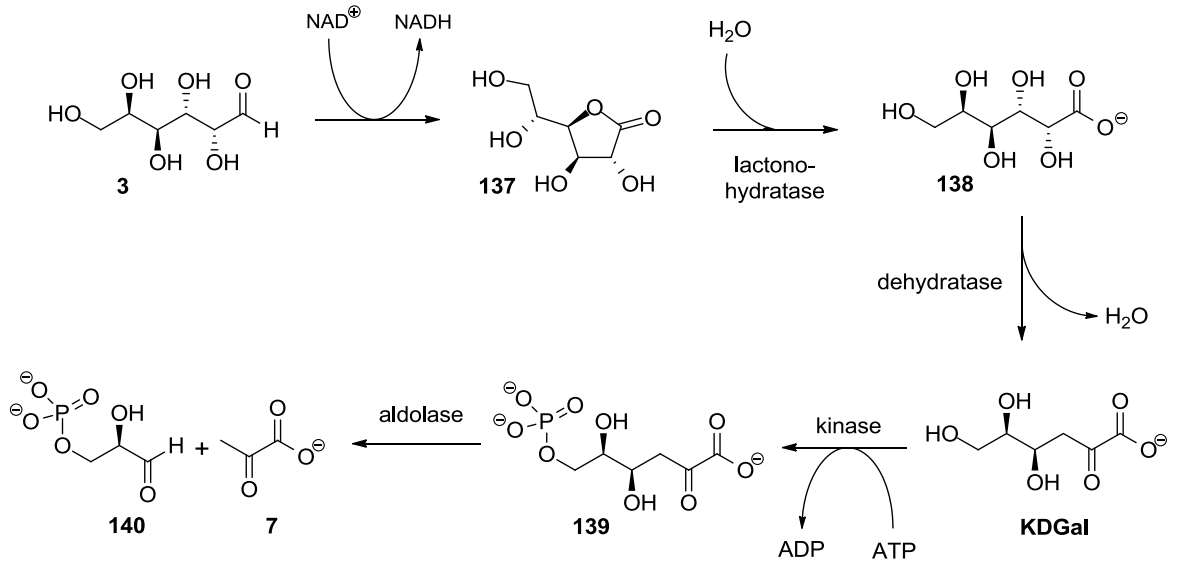
Scheme 21 *Kang's synthesis of Neu5Ac **18** from non-sugar starting materials*



1.7 Bio-occurrence of C6-2-Keto-3-Deoxy-Ulosonic Acids

D-KDG and **D-KDGal** are key intermediates in the metabolism of D-glucose **2** and D-galactose **3** by many organisms. In the non-phosphorylative Entner-Doudoroff pathway, found in fungi and archaea, a dehydratase catabolises D-gluconate **4** (or D-galactonate **5**) to **D-KDG** (or **D-KDGal**) respectively, before they are catabolised further by an aldolase to afford D-glyceraldehyde **6** and pyruvate **7** (Section 1.2). In the classical Entner-Doudoroff pathway,[92-93] the 6-phosphorylated form of **D-KDG** is an intermediate that is catabolised by KDPG aldolase to give D-glyceraldehyde **6** and pyruvate **7**. Meanwhile, the DeLey-Doudoroff pathway that catabolises D-galactose **4** to pyruvate **7,** which was first discovered in a strain of *Pseudomonas sacharophila* and has since been found to operate in many other bacteria,[18,94-96] also generates **D-KDGal** as an intermediate (Scheme 22). In detail, this bacterium oxidises D-galactose **3** in the presence of NAD^+ to D-galactono-γ-lactone **137**, which is enzymatically hydrolyzed to galactonate **138**. Acid **138** is subsequently dehydrated to **D-KDGal** by a galactonic acid dehydratase, then phosphorylated before being catabolised to pyruvate **7** and D-gylceraldehyde-3-phosphate **140**.

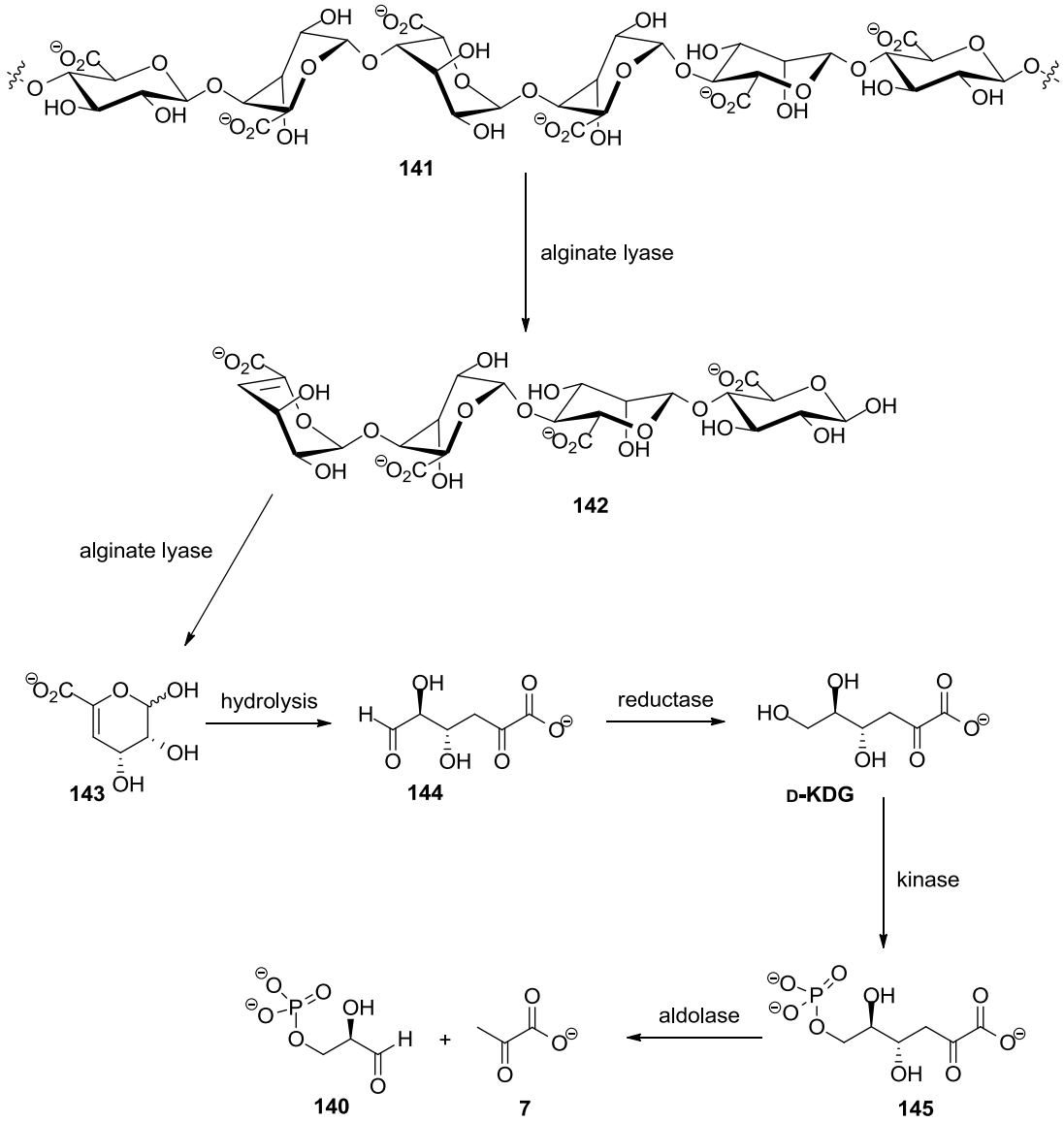
Scheme 22 *DeLey-Doudoroff metabolism of* ***D-KDGal*** *found in certain bacteria*



D-KDG has been shown to be an intermediate in the metabolism of alginate **141** by *Sphingomonas sp.* strain A1, a Gram-negative bacterium (Scheme 23).[97] Alginate **141**, which is a polysaccharide made up of repeating (1→4) β-D-mannuronate and α-L-guluronate residues found naturally in bacteria, algae and seaweed, is first transferred to

the cell's cytoplasm where alginate lyases degrade it into its constituent monosaccharides. These are then non-enzymatically converted into 4-deoxy-L-erythro-5-hexulose uronic acid (DEH) **144**, and this sugar is reduced to **D-KDG** by DEH reductase. The **D-KDG** produced is then phosphorylated to afford **145** that is then cleaved to pyruvate **7** and glyceraldehyde-3-phosphate **140** *via* a *retro*-aldol reaction.

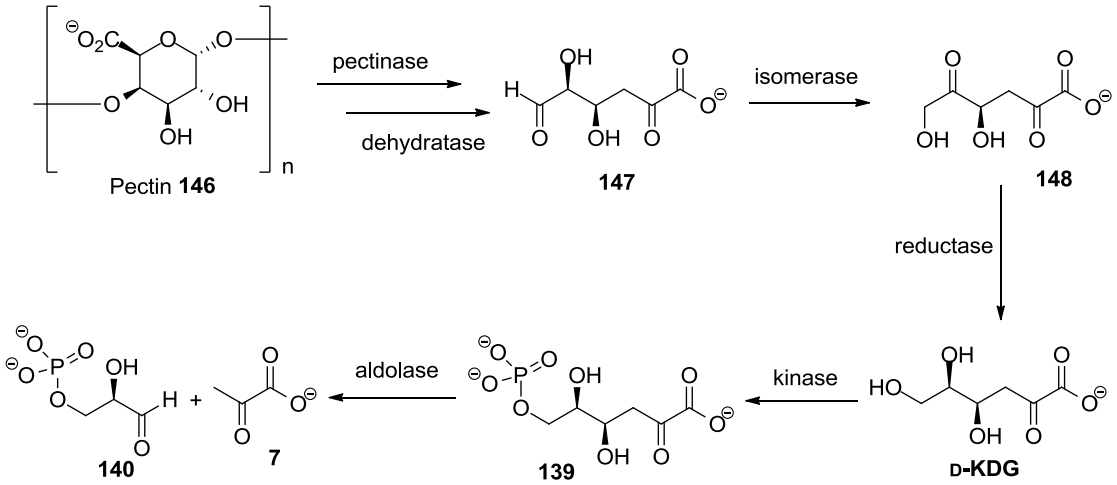
Scheme 23 *Metabolism of alginate by Sphingomonas sp. strain A1*



D-KDG has also been shown to be both an intermediate in the catabolism of pectin and a regulator of gene expression for the catabolism of pectin **146** by the bacterium *Erwinia*

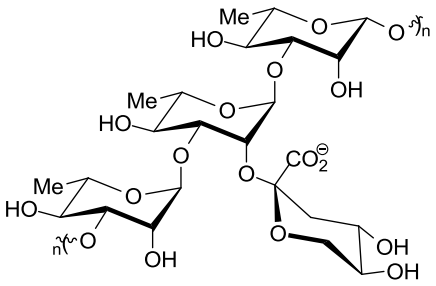
chrysanthemi (Scheme 24).[98-100] This bacterium causes soft-rot disease by degradation of plant cell walls, which leads to death of the plant tissue and provides nutrients for bacterial growth. Robert-Baudouy and co-workers have shown that most of the genes involved in pectin degradation, which is the first step in the degradation of the plant cell walls, are controlled by a single down-regulated gene. When **D-KDG** interacts with this regulatory gene it induces pectin catabolism pathways.[101]

Scheme 24 *Pathway for pectin catabolism by E. chrysanthemi*



It is well known that higher 2-keto-3-deoxy-ulosonic acids play an important structural role in specific prokaryotes as constituents of lipopolysaccharides (Section 1.6). **D-KDGal** has been identified as a constituent of the extracellular polysaccharides of *Azotobacter vinelandii*, where it is attached to rhamnose by a β-(2→2) gylcosidic bond (Figure 5).[102-103] It is thought that these extracellular lipopolysaccharides play a role in intercellular communication affecting cell proliferation, cell-cell adhesion and cell migration.

Figure 5 *Lipopolysaccharide containing **D-KDGal** fragment from cell wall of Azotobacter vinelandii*

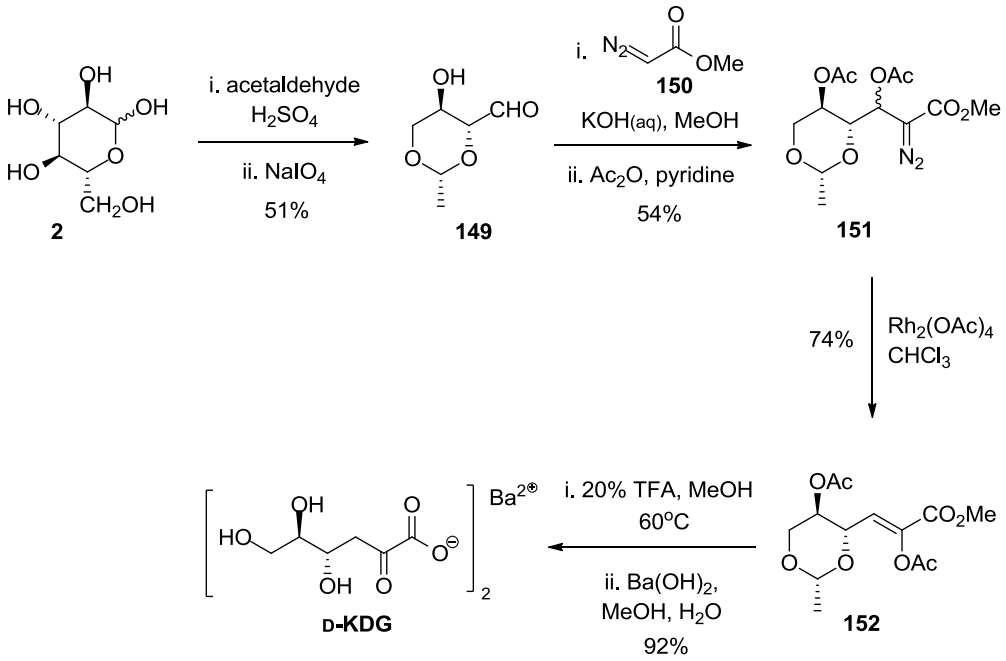


1.8 Previous Syntheses of D-KDG and D-KDGal

1.8.1 Rhodium-Mediated Carbenoid Rearrangement Methology

Rhodium mediated decomposition of *β*-acetoxy-*α*-diazoesters has been used by the Herrera group to prepare higher 2-keto-3-deoxy-ulosonic acids (Section 1.6.7) and for the synthesis of **D-KDG** (Scheme 25).[104] Thus, protected aldehyde **149** was prepared in two steps from D-glucose **2**, and treated with methyl diazoacetate **150** to give *β*-acetoxy-*α*-diazoester **151** after acetyl protection of the two hydroxyl groups. Addition of a catalytic quantity of rhodium acetate successfully induced rearrangent to give enol ester **152**, which was subjected to acidic conditions and isolated as the barium salt of **D-KDG**. The overall yield for this synthesis was considerably lower than that achieved by the Herrara group for the synthesis of KDN **17** (19% compared to 85%), which was due in part to the addition of methyl diazoacetate to aldehyde **149** affording just a 54% yield of product.

Scheme 25 *Herrera synthesis of* ***D-KDG***

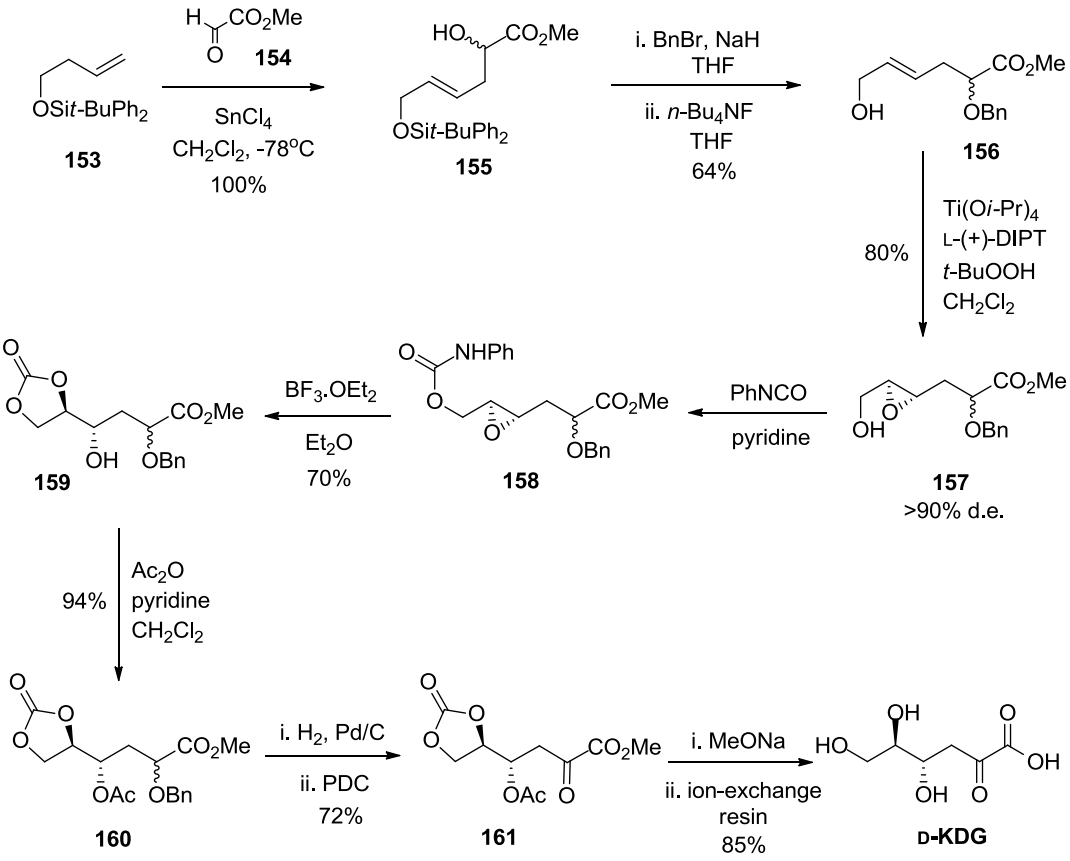


1.8.2 Synthesis from Non-Sugar Based Starting Materials

Mikami and co-workers showed that a glyoxylate-ene reaction mediated by stannic tetrachloride was a suitable starting point for the synthesis of **D-KDG** from non-sugar

starting materials.[105] The initial ene-reaction of **153** with methyl glyoxalate **154** proceeded with excellent selectivity furnishing allylic alcohol **155** exclusively in the *E*-configuration (Scheme 26). After benzyl deprotection of the secondary hydroxyl group and silyl deprotection, a Sharpless epoxidation reaction on homoallylic alcohol **156** gave epoxide **157** with good stereoselectivity (>90% e.e.). Two-step epoxide ring-opening was then carried out by treatment of **157** with phenyl isocyanate followed by $BF_3.OEt_2$, inducing ring closure to give carbonate **159**. *O*-Acetylation, *O*-debenzylation and oxidation with PDC gave α-keto ester **161** that was hydrolysed to complete the synthesis over eleven steps with an overall yield of 21%.

Scheme 26 *Mikami synthesis of* ***D-KDG***

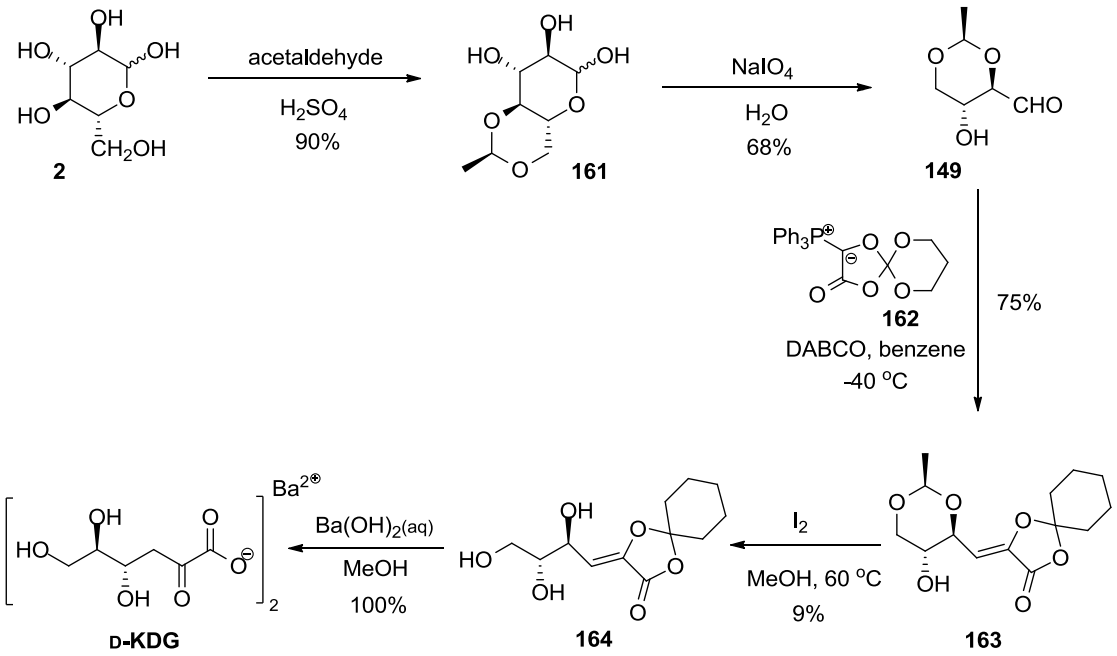


1.8.3 Horner-Wadsworth-Emmons Methodology

Ramage and co-workers prepared aldehyde **149** in a similar way to the Herrera group, but instead of using methyl diazoacetate to introduce the α-keto acid group they employed their Wittig methodology to introduce the masked α-keto acid of **D-KDG** (Scheme 27).[53]

Unfortunately, acidic deprotection, which had been successful in the synthesis of KDO **16** (Scheme 8), led to degradation. However, they found that treatment with a methanolic solution of iodine (1% v/v) gave the deprotected triol **164**, but in a very low 9% yield, so that final treatment with barium hydroxide afforded **D-KDG** in a very disappointing overall 4% yield.

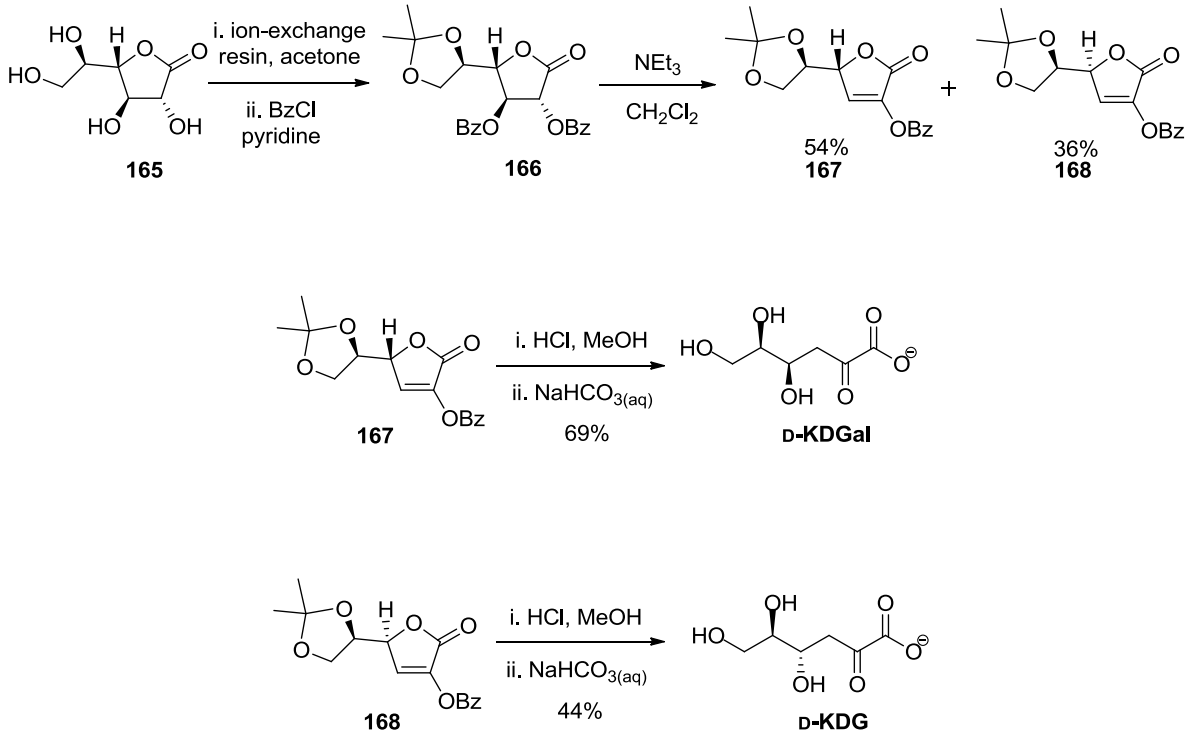
Scheme 27 *Ramage synthesis of* ***D-KDG***



1.8.4 β-Elimination Methodology

Thiem and Limberg have shown that triethylamine catalysed *β*-elimination of benzoyl protected lactone **166** allows access to both **D-KDGal** and **D-KDG** in arguably the most concise and high yielding route to date (Scheme 28). Starting from lactone **165**, the selective protection of the hydroxyl groups in the side chain was followed by benzoylation of the remaining hydroxyl groups to furnish **166**. Treatment with a catalytic quantity of triethylamine led to β-elimination *via* an E1cB mechanism, with concomitant epimerisation at the δ-position to give a mixture of epimers **167** and **168**.[106] However, purification by column chromatography allowed both epimers to be isolated and subsequent hydrolysis furnished **D-KDGal** and **D-KDG** in an impressive 32% and 14% overall yield respectively over just five steps.

Scheme 28 *Thiem and Limberg synthesis of* ***D-KDGal*** *and* ***D-KDG***



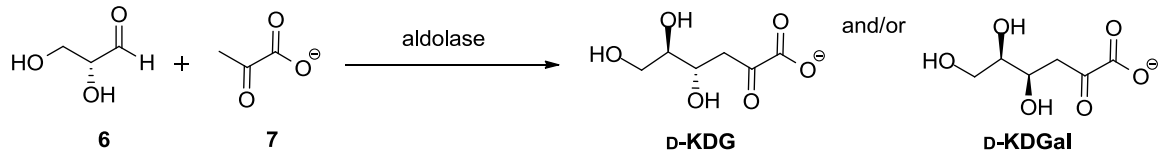
165 166 167 168 **D-KDGal** **D-KDG**

1.8.5 Enzymatic Methodology

Aldolases from both bacteria and filamentous fungi have been used to prepare **D-KDG** and **D-KDGal** (Scheme 29).[107-116] Thus Augé and Delest found that when the filamentous fungus *Aspergillus niger* was grown on 2% D-gluconate **4** as the sole sugar source, it produced high concentrations of KDG aldolase.[109] The enzyme was partially purified by heat treatment of the ground cells followed by precipitation with acetone. Incubation of D-glyceraldehyde **6** and pyruvate **7** (1.1 equivalents), with the isolated KDG aldolase, afforded a diastereomeric mixture of **D-KDG** and **D-KDGal** in a ratio of 87:13. More recently they have reported that crude cell extracts from the filamentous fungus *Aspergillus terreus* catalysed the aldol reaction of a range of aldehydes with pyruvate giving high levels of stereoselectivity.[108] Using this method, D-glyceraldehyde **6** was incubated with pyruvate **7** (1 equivalent) and the crude cell extracts of *A. terreus* to afford **D-KDG** in 100% d.e. Meanwhile Wong and co-workers have used KDO aldolase from the Gram-positive bacterium *Aureobacterium barkerei* and found that the enzyme promotes attack of pyruvate **7** at the *Re* face of aldehydes,[82] therefore giving complementary stereoselectivity to the fungal aldolase studied by Augé and Delest. The enzyme was

partially purified by ammonium sulfate precipitation of crude cell extracts, and then incubated with D-glyceraldehyde **6** and pyruvate **7** to give **D-KDGal** (>97% d.e.). 2-Keto-3-deoxy-6-phosphogluconate aldolase (KDPG aldolase) has also been employed to catalyse the reaction of non-phosporylated substrates by Toone and co-workers.[117] They incubated KDPG aldolase D-glyceraldehyde with pyruvate to give **D-KDG** stereoselectively (>97% d.e.) in 30% isolated yield. More recently they improved the catalytic activity of KDPG aldolase from *E. coli* for the reaction of D-glyceraldehyde **6** and pyruvate **7**, by mutation of key active site residues.[113]

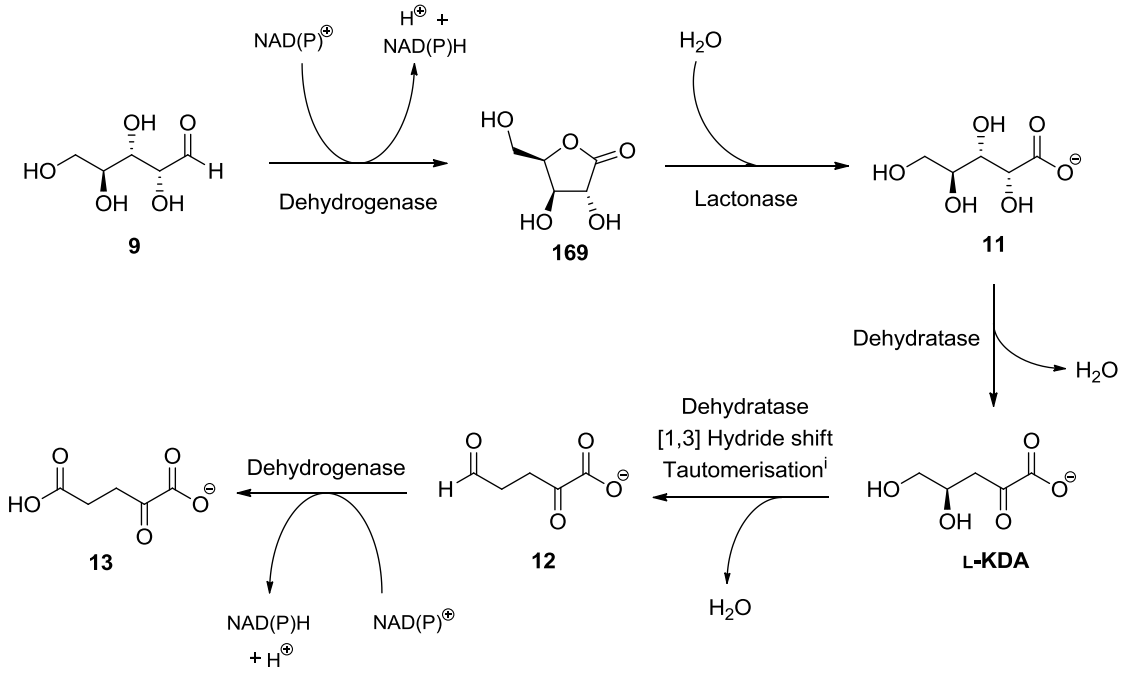
Scheme 29 *Aldolase synthesis of* ***D-KDG/D-KDGal***



1.9 Bio-occurrence of C5-2-Keto-3-Deoxy-Ulosonic Acids

It has been well documented that **D-KDX** and **L-KDA** are metabolites of the five carbon aldose sugars D-xylose **8** and L-arabinose **9** in some fungi, bacteria and archaea. [101,6,118] Makino and co-workers have shown that when *Azospirillum brasiliense* is grown on L-arabinose **9** as the sole carbon source, that L-arabinose **9** is catabolised to 2-oxoglutarate **13** *via* **L-KDA** as an intermediate (Scheme 30).[118] In this pathway, L-arabinose **9** is first oxidised to L-arabino-γ-lactone **169** by a NAD(P)$^+$-dependant dehydrogenase. This lactone **169** is then cleaved by a lactonase to afford L-arabonate **11** that is then dehydrated to **L-KDA**. Further dehydration affords 2-oxoglutarate semialdehyde **12** that is then converted by a NAD(P)$^+$-dependant dehydrogenase to 2-oxoglutarate **13**, which is utilised by the bacterium in the citric acid cycle, for the synthesis of glutamine, or for the transport of nitrogen. Evidence for this pathway has also been found for *Pseudonomas fragi*,[119] *Pseudomonas saccharophila*, [118,120-121] *Pseudomonas aeruginosa*,[122] *Rhibium japonicum*,[123-125] *Herbaspirillum seropedicae*, [126] *Aspergillus ustus*,[114,127] and *S. solfataricus*.[6] Despite the numerous references to **D-KDX** and **L-KDA** in the literature, there are few reported syntheses, which is perhaps due to the reported instability of these sugars to acidic and basic conditions where they have been observed to degrade at pH 2.0 at room temperature over a period of days.[128-129]

Scheme 30 *Catabolism of L-Arabinose* ***9*** *by Azospirillum brasiliense and Pseudomonas saccharophila*



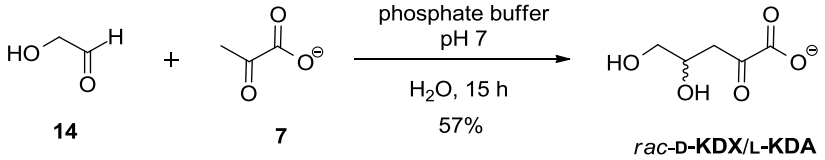
i. see Scheme 3

1.10 Previous Syntheses of C5-2-Keto-3-Deoxy-Ulosonic Acids

1.10.1 Nucleophilic Methodology

There is only one instance of the chemical synthesis of the C5-2-keto-3-deoxy-ulosonic acids **D-KDX** and **L-KDA**. In this example pyruvic acid **7** and glycolaldehyde **14** were allowed to react under neutral conditions to furnish a racemic mixture of **D-KDX** and **L-KDA** in 85% yield (Scheme 31).[120,130-131]

Scheme 31 *Stoolmiller synthesis of* ***D-KDX*** *and* ***L-KDA***

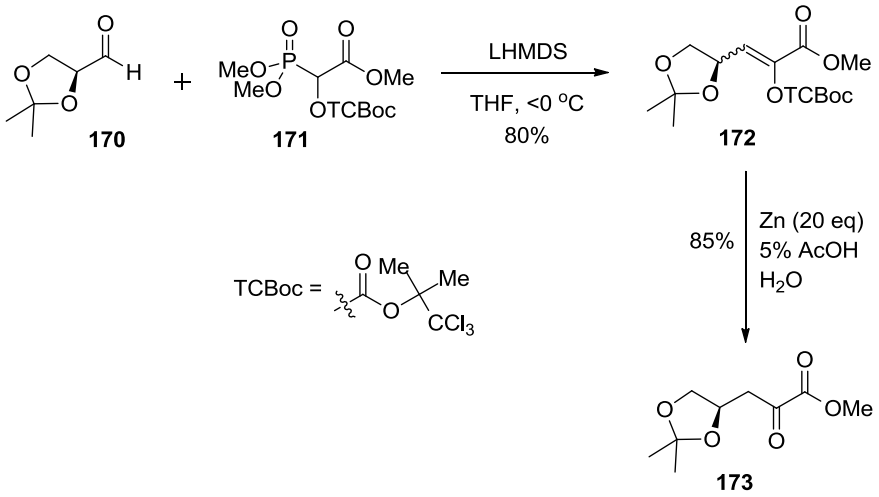


1.10.2 Horner-Wadsworth-Emmons Methodology

Horne and co-workers have shown that the HWE methodology can also be applied to the synthesis of **173**, the protected methyl ester derivative of **L-KDA** (Scheme 32).[132] They reacted the lithium anion of phosphonate ester **171** with L-glyceraldehyde acetonide **170**

to furnish **172** as a mixture of *cis*- and *trans*-geometrical isomers. Reductive elimination of the trichloro-*tert*-butoxyl carbonate group was achieved by treatment of an AcOH/H_2O solution of **172** with 20 molar equivalents of zinc dust and purification by silica gel column chromatography, which afforded α-keto methyl ester **173** in overall 67% yield. The authors did not proceed to deprotect the diol moiety or hydrolyse ester **173** to **L-KDA**.

Scheme 32 *Horne HWE methodology for the synthesis of* ***L-KDA*** *derivative*



1.10.3 β-Elimination Methodology

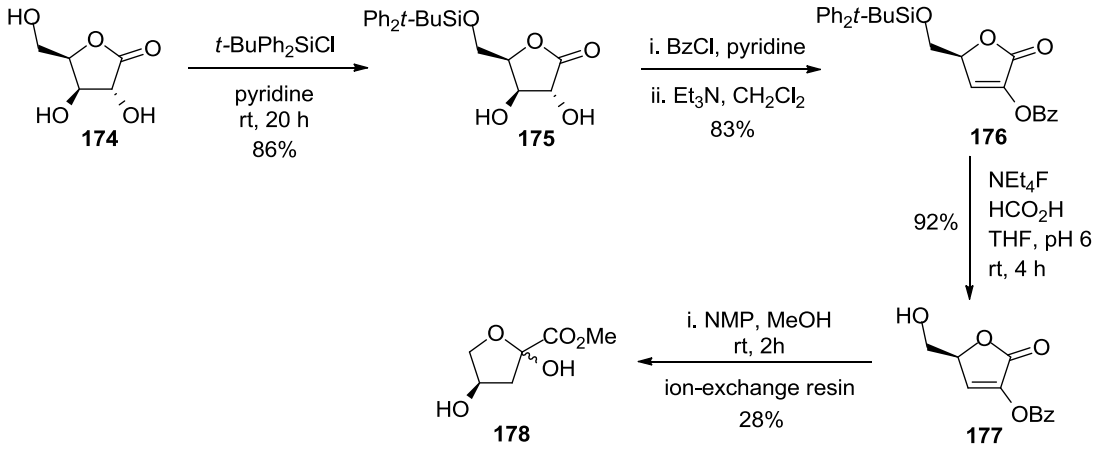
Theim and co-workers have also applied their β-elimination methodology to the synthesis of the methyl ester derivative **178** of **L-KDA** (Scheme 33).[106] Selective silyl protection of the primary hydroxyl group of L-arabinono-1,4-lactone with *tert*-butylchlorodiphenylsilane furnished **175**, followed byenzoylation of the remaining hydroxyl groups and treatment of the crude product with Et_3N resulted in efficient β-elimination to afford 3-deoxy-2-enolactone **176**. The silyl protecting group was then removed using a buffered THF solution of tetrabutylammonium fluoride to give **177**. The authors found that standard acidic deprotection procedures led to decomposition, which they concluded was a result of instability of the sodium salt of **L-KDA**. This problem was avoided by employing a mild debenzoylation method involving treatment of a THF/methanol solution of **177** with *N*-methylpyrrolidine, subsequent acidification with Amberlite IR-120 (H^+) resin afforded the methyl ester derivative **178** of **L-KDA** in overall 18% yield.

1.10.4 Enzymatic Methodology

Meanwhile a few groups have used enzymic methodology for the synthesis of **D-KDX** and **L-KDA**, although the products were not fully characterised and their structure inferred from derivatisation studies carried out *in situ*.[120,133-135] Having studied the metabolism of L-

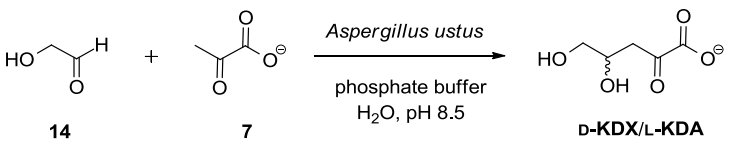
arabinose **9** to 2-ketoglutarate **13** by cell-free extracts of *Pseudomonas saccharophila* (Scheme 30), Abele and Stoolmiller employed L-arabonate dehydratase for the synthesis of **L-KDA**, which was isolated by paper chromatography.[120,133] **L-KDA** synthesised by this

Scheme 33 *Thiem β-elimination methodology synthesis of* ***L-KDA*** *derivative*



method appeared to be only 80% pure when subjected to an enzyme assay. In a similar manner Frost and co-workers have used mutant *E. coli* cultures that express an L-arabonate dehydratase to convert L-arabinose **9** to **L-KDA** before reacting it *in situ* to afford 1,2,4-butanetriol.[134] Abdel-Fatah and Elshafei have demonstrated that 2-keto-3-deoxy-L-arabonate aldolase, present in cell-free extracts of *Aspergillus ustus*, can be used for the synthesis of **L-KDA** from glycolaldehyde **14** and pyruvate **7**, however the entantiomeric excess of the product was not determined (Scheme 34).[135] In this case the **L-KDA** synthesised was not isolated being characterised indirectly using a thiobarbituric acid assay, and used to compare the enzyme efficacy towards various aldehyde and acid substrates.

Scheme 34 *Aspergillus ustus aldolase mediated synthesis of* ***L-KDA***



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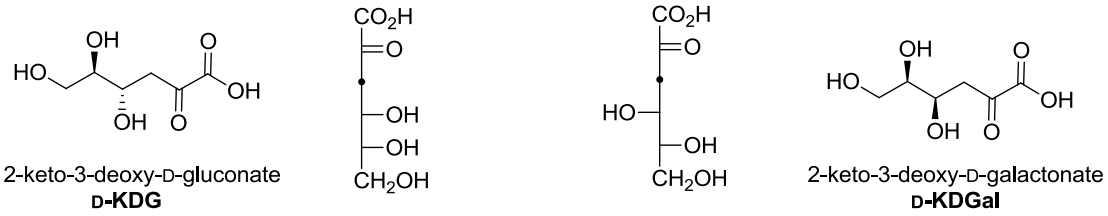
Chapter 2

2.1 Introduction

Two chemical syntheses of **D-KDG** were initially investigated with the expectation that any successful methodology developed could then be applied to the synthesis of **D-KDGal**. The first unsuccessful approach involved introducing the α-keto acid functionality by oxidation of the terminal 1,2-diol of a suitably protected C6-substrate. The second successful strategy consisted of a Horner-Wadsworth-Emmons (HWE) reaction to give a silyl-enol ether that would then be globally deprotected to afford enantiomerically pure **D-KDG**. Once the two target sugars were synthesised, their structure and the effect of temperature on their isomeric composition in solution would be investigated, before they were then employed as substrates for the directed evolution of KDG aldolase from *S. solfataricus*.

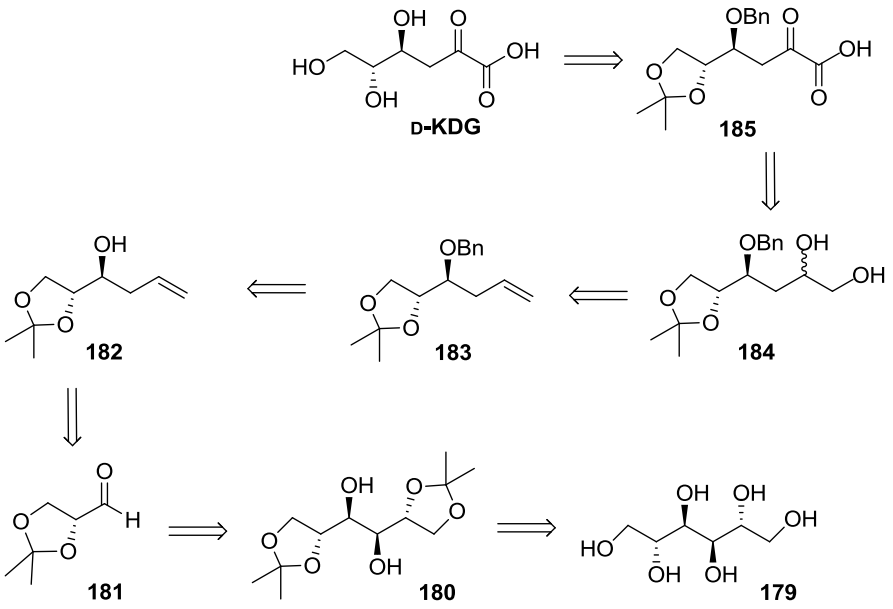
Figure 6 *Structures of **D-KDG** and **D-KDGal***



2.2 Strategy A: Oxidative Synthetic Strategy for the Synthesis of D-KDG

Initially an oxidative strategy was envisaged for the synthesis of the target sugars (Scheme 35). It was proposed that **D-KDG** could be synthesised from the chiral pool starting material D-mannitol **179** by acetonide protection of the terminal diol functionality and oxidative cleavage of the central carbon-carbon bond of *bis*-acetonide **180**. Diastereoselective metal mediated allylation of **181** would then form alcohol **182**, which would be protected as benzyl ether **183** before dihydroxylation and oxidation would give α-keto acid **185**. Deprotection of both the acetonide and benzylic protecting groups would then give **D-KDG** in seven steps. If this synthetic strategy was successful, a related protocol would then be applied to the synthesis of **D-KDGal** and other 2-keto-3-deoxy-ulosonic acids.

Scheme 35 *Oxidative retro-synthetic strategy for the synthesis of* ***D-KDG***

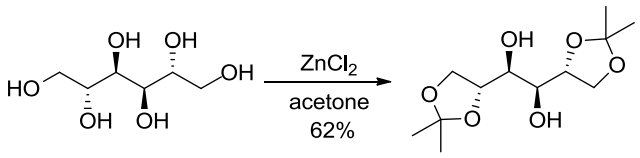


2.2.1 *Bis*-Acetonide Protection of D-Mannitol

D-Mannitol **179** has been used as the starting point for the synthesis of many chiral compounds, because it is a readily available and inexpensive chiral pool reagent. A number of different protocols are available for the selective protection of the different hydroxyl groups of D-mannitol, including the use of: benzaldehyde (1,3:4,6 protection);[1] 2,3-butadione (1,2:5,6 protection);[2] or trityl chloride (1:6 protection).[3]

For the synthesis of **D-KDG** the acetonide protecting group was chosen to achieve 1,2:5,6 protection. This reaction has been studied systematically by the Kuszmann group using GC-MS and NMR spectroscopic analysis.[4] They showed that the choice of reagents, solvent and reaction time were crucial for achieving good yields. Prolonged reaction times favoured the formation of tri-acetonide products whilst some solvent/reagent combinations gave mono-acetonide and various isomeric *bis*-acetonide impurities. Following their optimised method, D-mannitol **179** was reacted with anhydrous zinc (II) chloride in dry acetone for 22 hours at 15-20 °C. The crude product was purified by recrystallisation from carbon tetrachloride to give the desired product **180** as a white crystalline solid in 62% yield (Scheme 36).

Scheme 36 *Bis-acetonide protection of D-mannitol*

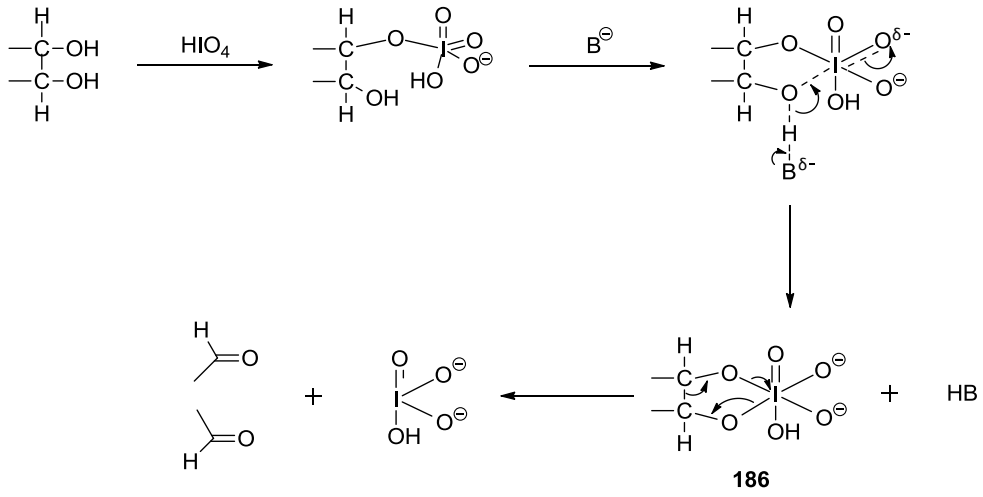


179 **180**

2.2.2 Oxidative Cleavage of D-Mannitol *Bis*-Acetonide

Cleavage of the carbon-carbon bond of 1,2-diols can be achieved with a variety of reagents, however, there was clear literature precedent for the use of sodium periodate to cleave *bis*-acetonide **180**. The mechanism for the sodium periodate cleavage of 1,2-diols, according to Buist,[5] involves initial formation of a cyclic periodate ester **186**, which then decomposes to give two molecules of aldehyde (Scheme 37).

Scheme 37 M*echanism for sodium periodate cleavage 1,2-diol*

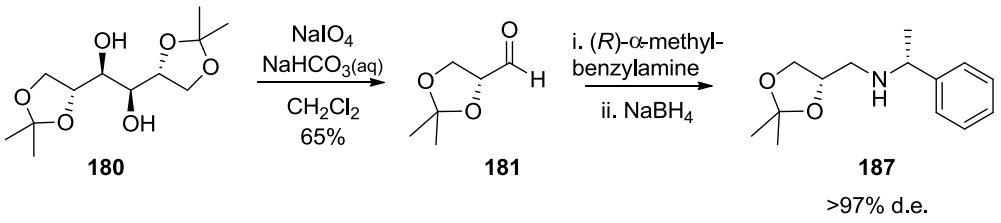


186

A sodium bicarbonate mediated sodium periodate protocol, developed by Schmid and co-workers, was followed (Scheme 38).[6] The base was added to neutralise any acetic acid by-product formed, which is believed to cause an increase in product polymerisation. After purification of the crude product by distillation, the desired aldehyde **181** was obtained in 65% yield. The enantiomeric excess was initially confirmed by polarimetry that gave $[\alpha]_D^{27}$ +60.4 (*c* 2.25, EtOAc). There is some disagreement in the literature over

the exact optical rotatory power of **181**, with optical rotation values ranging from $[\alpha]_D$ = +54 to $[\alpha]_D$ = +80.[7-10] Since obtaining **181** in an enantiopure form was crucial for the successful synthesis of enantiomerically pure **D-KDG**, further proof of the enantiomeric excess of **181** was acquired by chiral derivatisation. Accordingly **181** was reacted with (*R*)-α-methylbenzylamine, reduced *in situ* with sodium borohydride and analysed by ^{1}H NMR spectroscopy. This showed that the diastereomeric excess of the secondary amine product **187** was >97% inferring an enantiopurity for **181** of >97% e.e. D-Glyceraldehyde acetonide **181** was reacted immediately, as it is known to be unstable and prone to racemisation, even when stored under an inert atmosphere in a refrigerator.

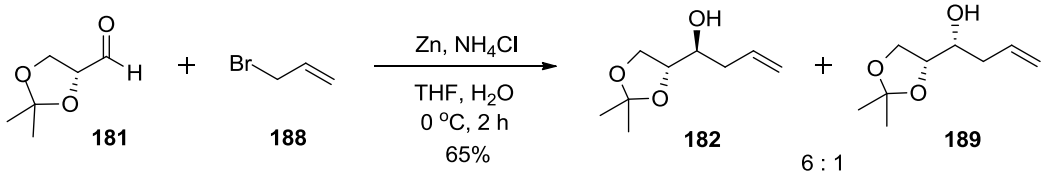
Scheme 38 *Schmid base mediated sodium periodate methodology for the synthesis of* D*-glyceraldehyde acetonide* ***181***



2.2.3 Metal-Mediated Allylation of D-Glyceraldehyde Acetonide

Methodology for the addition of metal-allyl species to aldehydes and ketones is an important research area, offering a valuable alternative to conventional aldol methodologies for creating homoallylic alcohols. Luche's aqueous procedure was followed, whereby aldehyde **181** was treated with excess allyl bromide **188** and zinc dust in a $NH_4Cl_{(aq)}$/THF reaction medium (Scheme 39).[11]

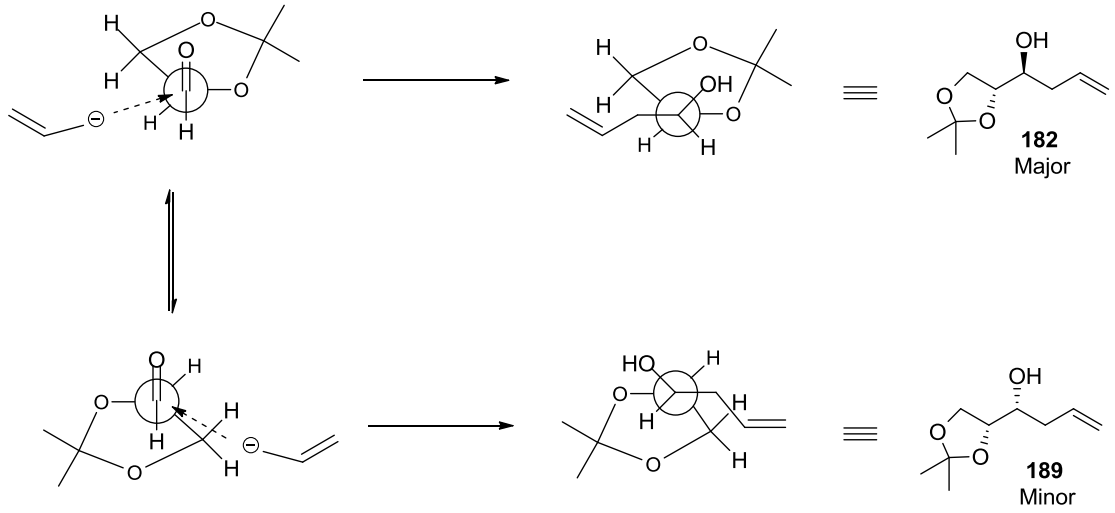
Scheme 39 *Zinc catalysed allylation of* D-glyceraldehyde acetonide



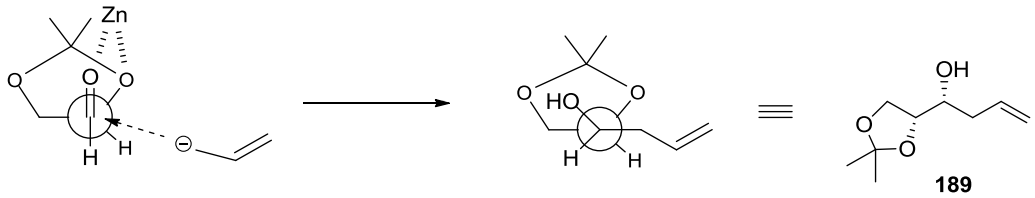
The diastereoselectivity of the reaction (*anti*/*syn* 6:1) was determined by reaction of the crude product with benzyl bromide using KO*t*-Bu as base, followed by comparison of the

^{1}H NMR spectrum of the products with literature data, *vide infra*. Interestingly, the major *anti* product **182** was obtained as predicted by the Felkin-Anh model (Scheme 40), rather than a chelated-Cram model (Scheme 41). The absence of chelation is probably due to water solvation of the zinc ions preventing metal chelation to the carbonyl group of **181**.

Scheme 40 *Felkin-Anh model for stereoselective addition of allyl group to* D*-glyceraldehyde acetonide*



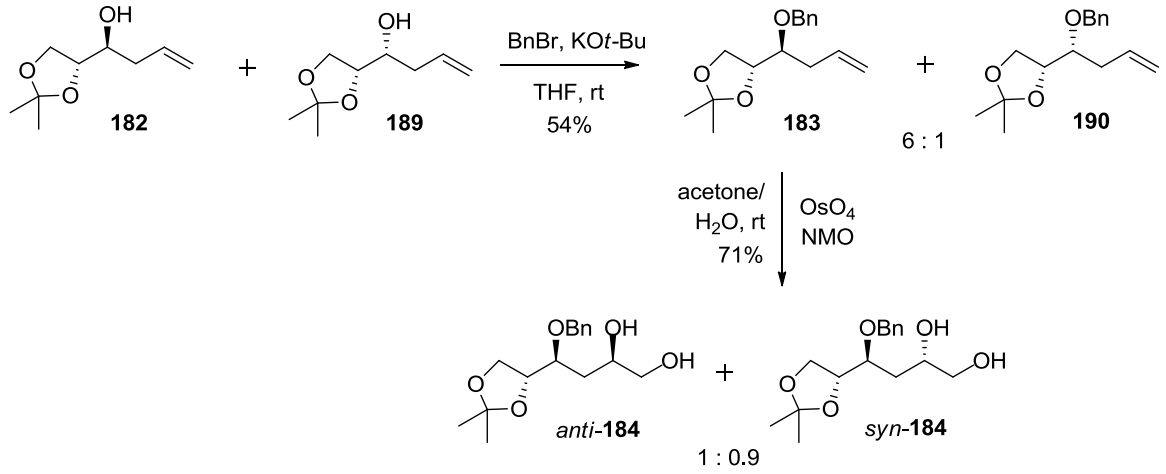
Scheme 41 *Chelation-Cram model for addition of allyl group to* D*-glyceraldehyde acetonide*



2.2.4 Benzyl Protection and Dihydroxylation

Alcohols **182** and **189** were protected as their corresponding benzyl ethers and the two diastereomers **183** and **190** (*anti*/*syn* 6:1) effectively separated by silica gel column chromatography (Scheme 42). Comparing the ^{1}H and ^{13}C NMR spectra to literature values it was possible to assign the main diastereomer as having an *anti*-configuration.[12] The *anti*-diastereomer **183** was then dihydroxylated *via* treatment with OsO_4 and NMO to give diol **184**,[13] as a 1:0.9 mixture of diastereomers, in good yield after purification.

Scheme 42 *Benzyl protection and dihydroxylation of alcohol-acetonides **182** and **189***



2.2.5 Attempted Oxidation of 1,2-Diol 184

Oxidation of the 1,2-diol fragment of **184** to its corresponding α-keto acid (or ester) required the use of a mild oxidant, since cleavage of the terminal C-C bond could potentially occur under oxidative conditions to give a C5-carboxylic acid with loss of carbon dioxide. Initially a range of literature oxidation protocols were trialled on 1,2-octanediol **191**, which led to varied amounts of 2-oxooctanoic acid **192**, heptanoic acid **193** and starting material **191** (Table 5).

Scheme 43 *Oxidation of 1,2-octanediol **191***

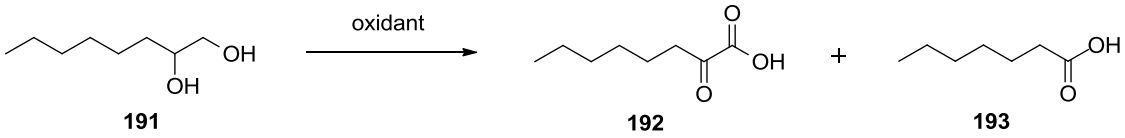
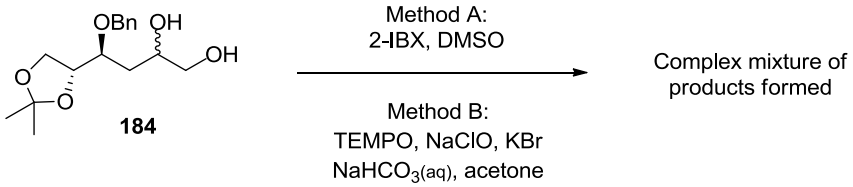


Table 5 *Conditions screened for the oxidation of 1,2-diol **191** to α-keto acid **192***

Entry	Oxidant (molar equivalents)	Co-oxidant (molar equivalents)	Result
1[14]	2-IBA (0.6)	Oxone (5.4)	**193** (54%)
2[14]	2-IBA (0.2)	Oxone (1.8)	**193** (71%)
3[15]	IBX (1.5)	-	**191** + impurities
4[15]	IBX (4.0)	-	**192** (65%)
5[16]	TEMPO (0.02)	TCCA (2)	**192** + **193**
6[17]	TEMPO (2.2)	NaClO (3.6)	**192** (90%)

Two successful methods, for the oxidation of 1,2-octanediol **191** were identified, using either an excess of 2-iodoxybenzoic (IBA) acid or tetramethylpiperidine *N*-oxide (TEMPO)/sodium hypochlorite. These reagents were employed for the oxidation of diol **184**, but the desired α-keto acid **185** could not be observed in the ^{1}H NMR spectra of the complex mixture of products formed for either method. ^{1}H NMR and mass spectroscopic analysis revealed the disappearance of starting material **184** and also some evidence of debenzylation. Variation of the reaction parameters (temperature, time and molar equivalents of oxidants and co-oxidants) of these oxidation reactions gave no evidence of any successful oxidation to α-keto acid products.

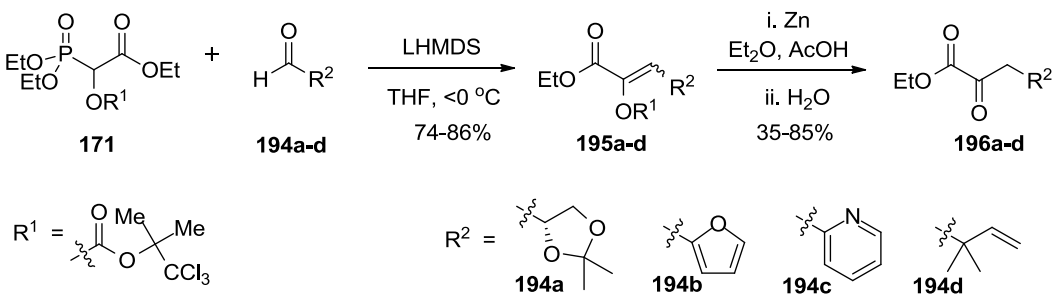
Scheme 44 *Failed oxidation of 1,2-diol **184***



2.3 Strategy B: Horner-Wadsworth-Emmons for the Synthesis of D-KDG

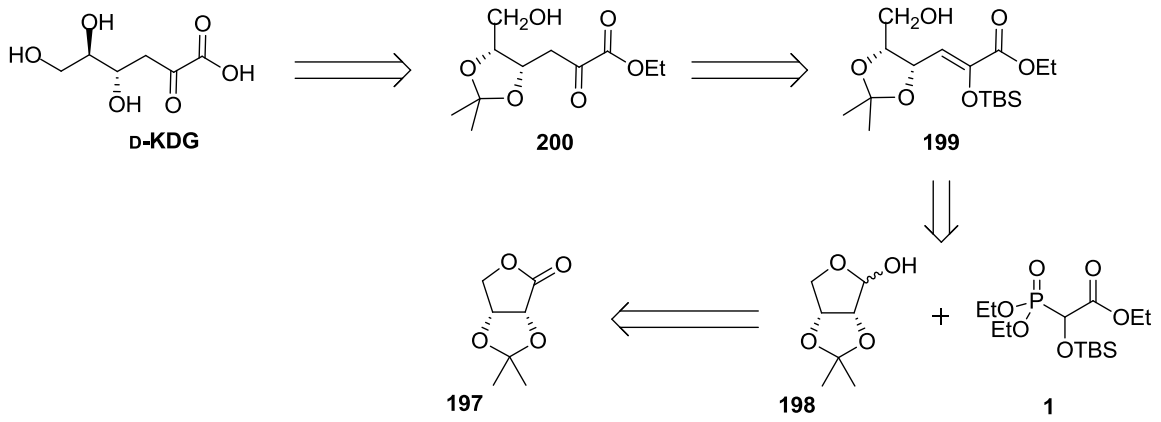
A careful search of the literature revealed that Thompson and co-workers had demonstrated that trichloro-*tert*-butyloxy carbonate protected phosphonate ester **171** could act as an acyl anion equivalent when reacted with aldehydes (Scheme 45).[18] This HWE reaction was successful for reacting a range of acid sensitive aldehydes **194a-d** with the enol-ether products **195a-d** being deprotected in high yield by treatment with zinc dust to afford α-keto acid products **196a-d**.

Scheme 45 *HWE precedent for the synthesis of α-keto acids*



Other groups went on to apply this type of HWE methodology for the synthesis of the higher 2-keto-3-deoxy-ulosonic acids DAH **15**, KDO **16** and KDN **17** (see Sections 1.6.4 and 1.8.3). Inspired by this work, an alternative synthetic strategy for the synthesis of **D-KDG** was proposed, which aimed to introduce the key α-keto acid functionality in a masked form using a HWE reaction (Scheme 46). The convergent synthesis would involve HWE reaction of the anion of ethyl 2-((*tert*-butyldimethylsilyl)oxy)-2-(dimethoxyphosphoryl) acetate **1** with the known lactol **198**, followed by global deprotection to afford **D-KDG** in just four steps.

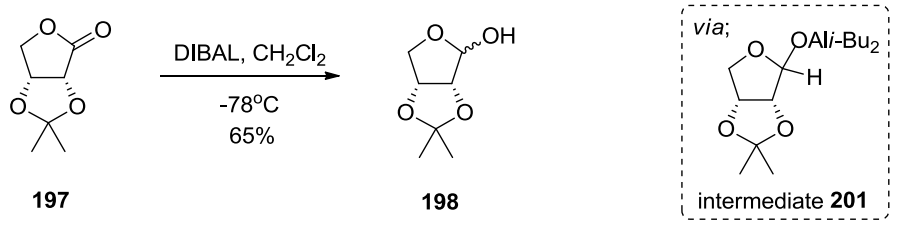
Scheme 46 *HWE retrosynthetic strategy for the synthesis of* ***D-KDG***



2.3.1 Synthesis of Lactol 198

The commercially available lactone **197** was a good starting point for the synthesis of **D-KDG** as it could be reduced in just one step to afford the desired lactol **198** (Scheme 47).[19] The reduction of lactones to lactols is a reliable transformation that does not usually result in over reduction to alcohols, as is often observed for the DIBAL reduction of esters. The success of the reaction is due to DIBAL generating a stable tetrahedral intermediate **201**, which affords lactol **198** on aqueous work-up at low temperature. In the case of lactone **197**, DIBAL reduction gave lactol **198** in good yield as a white solid that required no further purification.

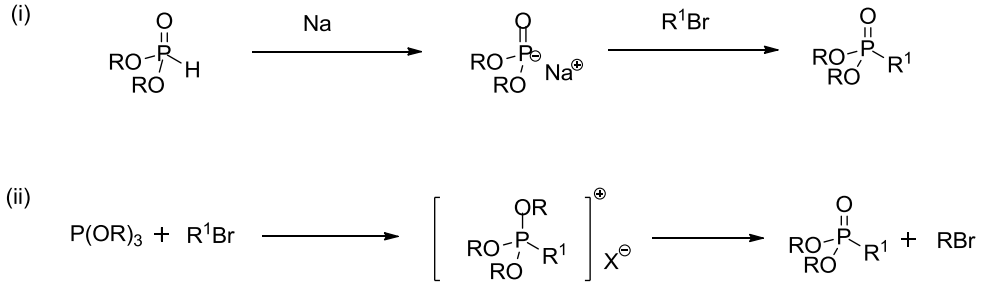
Scheme 47 *DIBAL reduction*



2.3.2 Phosphonate Ester Synthesis

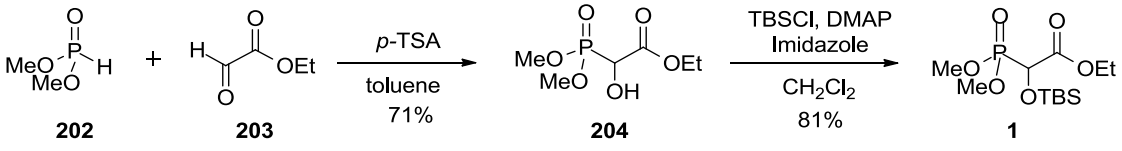
The Michaelis and Arbuzov reactions are the standard methods for the synthesis of phosphonates. The former involves the treatment of alkyl halides with alkyl metal derivatives of dialkyl phosphites (Scheme 48(i)), whilst the latter method involves reaction of a trialkyl phosphite with an alkyl halide (Scheme 48(ii)).

Scheme 48 *Michaelis and Arbuzov phosphonate ester syntheses*



The synthesis of phosphonate ester **204** has been previously reported using an unusual *p*-TSA catalysed coupling of a dialkyl phosphite **202** and ethyl glyoxalate **203** in refluxing benzene.[20] This methodology was repeated, substituting toluene for benzene, to successfully give phosphonate **204** in good yield and purity after silica gel column chromatography (Scheme 49). The hydroxyl functionality of **204** was then *O*-silyl protected using a standard protocol involving treatment with TBSCl, DMAP and imidazole to give ethyl 2-(dimethoxyphosphoryl)-2-hydroxyacetate **1** as a fragrant colourless oil.[21]

Scheme 49 *Synthesis of phosphonate ester **1***

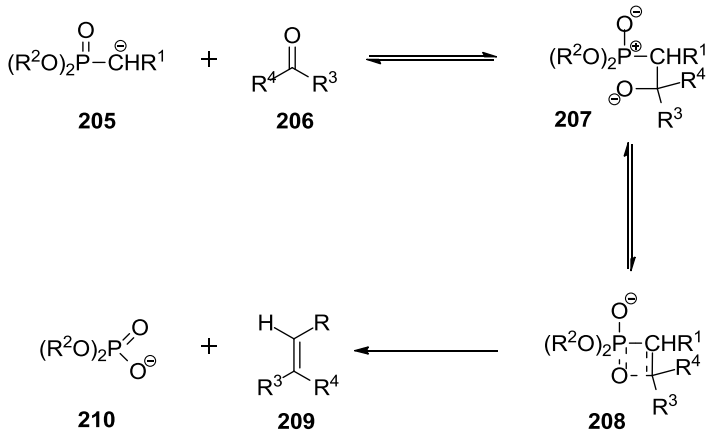


2.3.3 HWE Reaction for the Synthesis of Silyl-Enol Ether 199

The HWE and related Wittig reactions are powerful synthetic procedures. This is because the precursors are easily prepared, the reactions are high yielding and the alkene bond is normally generated in a regioselective and stereoselective manner. For the HWE

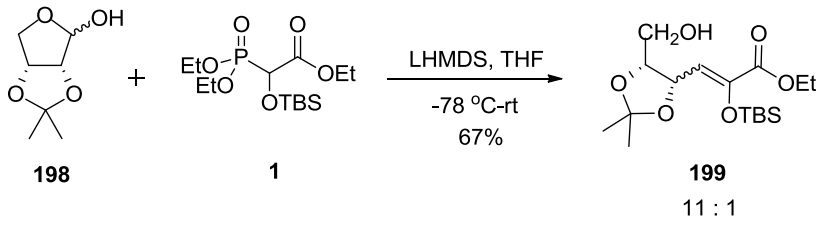
reaction, initial reversible nucleophilic attack of the deprotonated phosphonate anion **205** at the carbonyl bond of an aldehyde or ketone gives oxyanion **207** (Scheme 50). The oxyanion **207** then decomposes irreversibly with oxygen transfer to the phosphorous atom *via* a 4-membered cyclic transition state **208** to afford an alkene **209**. This process is thermodynamically favourable because of the strength of the phosphorous oxygen double bond being formed (P=O bond energy is 575 $kJmol^{-1}$). There are two key advantages that the HWE reaction has over the Wittig reaction. Firstly, the by-products of the HWE reaction are phosphonate salts **210**, which are water soluble and so can be easily removed by an aqueous work-up. Secondly, the HWE reaction can be conducted under milder conditions of base and temperature, which helps prevent racemisation of stereocentres in the α-position of the aldehyde, which was crucial for the successful synthesis of enantiopure **D-KDG**.[22]

Scheme 50 *HWE reaction mechanism*



Phosphonate ester **1** was deprotonated using LHMDS at -78 °C and then reacted with lactol **198** (Scheme 51). After allowing the reaction mixture to stir-warm to room temperature over 20 minutes, the crude product was isolated and purified by silica gel chromatography to give silyl-enol ether **199** as an inconsequential mixture of *E*/*Z*-geometrical isomers in 67% yield as a colourless oil.

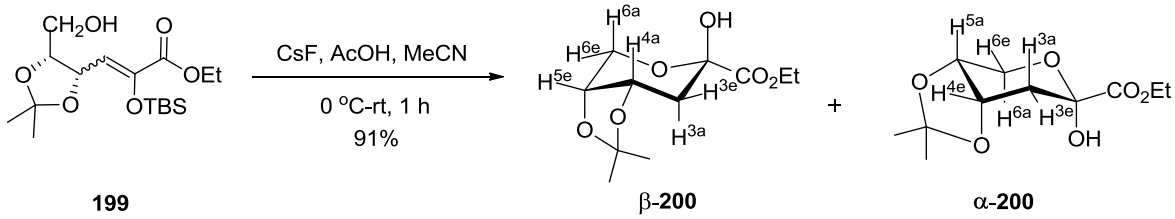
Scheme 51 *HWE step*



2.3.4 Silyl Protecting Group Deprotection

Silyl-enol ether **199** was efficiently deprotected using a standard method involving treatment of **199** with an acetic acid buffered acetonitrile suspension of cesium fluoride to give α-keto ester anomers β-**200** and α-**200** in high yield and good purity requiring no further purification (Scheme 52).[23] The structures of anomers β-**200** and α-**200** were proven by mass spectrometry and NMR spectroscopic data. Thus a mass ion with m/z 269.1001 corresponding to $[M+Na]^+$ (required 269.0996) showed that the TBS protecting group had been cleanly removed, which was confirmed by ^1H and ^{13}C NMR spectroscopic analysis that showed no TBS peaks, and that the alkene bond of **199** was no longer present. The ^{13}C NMR spectrum had no ketone carbonyl resonances present, but there were two resonances at 109 ppm as expected for the C(2) hemiacetal of the two anomers. In order to identify the conformation of the two isomeric forms of **200** it was first necessary to deduce whether the hydroxyl substituent at the anomeric centre was in the equatorial or axial positions. A survey of similar 2-keto-3-deoxy-ulosonic acid derivatives previously reported revealed that when the anomeric hydroxyl substituent is equatorial there is a significant downfield shift for the H^3 equatorial resonance whilst the H^3 axial resonance is unaffected.[24-27] A chemical shift difference for H^3 equatorial and axial resonances of 0.2-0.4 ppm indicates that the anomeric hydroxyl substituent is in the equatorial position. Both isomers of **200** have chemical shift difference between H^3 equatorial and axial resonances in this range (0.25 and 0.28 ppm), which indicates that β-**200** has a $^2C_5$ conformation and α-**200** has a $^5C_2$ conformation. Assignment of the major isomer of **200** as β-**200** followed from analysis of the coupling constants in the ^1H NMR spectrum (Table 6), since a large $J_{(3a4)}$, smaller $J_{(3e4)}$, and much smaller $J_{(6a5)}$ and $J_{(6e5)}$ were consistent with the structure of β-**200** where H^4 is axial and H^5 is equatorial.

Scheme 52 *Deprotection of the silyl group of **199***



199 β-**200** α-**200**

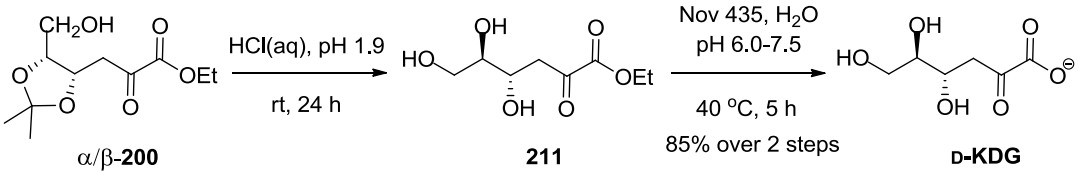
Table 6 *^1H NMR coupling constant of deprotection products β-**200** and α-**200** ($J_{H,H}$ in Hz)*

Compound	$J_{(3,3)}$	$J_{(3a,4)}$	$J_{(3e,4)}$	$J_{(6,6)}$	$J_{(6a,5)}$	$J_{(6e,5)}$
β-**200**	13.8	8.3	6.0	12.5	2.9	2.0
α-**200**	15.1	5.4	4.4	12.4	5.9	5.0

2.3.5 Acetonide and Ester Deprotection of α/β-200 for the Synthesis of D-KDG

Deprotection of the acetonide and ester groups of α/β-**200** was then carried out using the methodology painstakingly optimised for the synthesis of **D-KDX** (that will be fully discussed in the following chapter Section 3.2). Therefore, treatment of α/β-**200** with HCl(aq) at pH 1.9 followed by enzymatic hydrolysis of the ethyl ester **211** with Novozyme 435 (Nov 435) gave **D-KDG**, $[\alpha]_D^{25}$ -16.8 (c 0.24, D_2O at pH 6)(lit.[28] $[\alpha]_D^{20}$ -21.1 (*c* 5.67, H_2O) This furnished **D-KDG** in an excellent yield of 85%, with spectral data matching that reported in the literature (Scheme 53).

Scheme 53 *Final steps in the synthesis of* ***D-KDG***



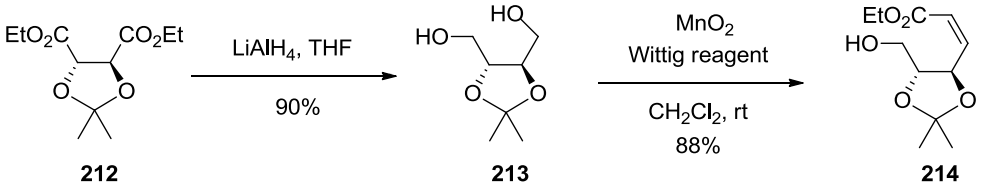
α/β-**200** **211** **D-KDG**

D-KDG had been synthesised over four steps in 34% overall yield from inexpensive commercially available starting materials using a HWE reaction and novel global deprotection methodology. Not only was this good methodology for the synthesis of **D-KDG**, but by varying the chiral aldehyde starting materials for the initial HWE reaction other 2-keto-3-deoxy-ulosonic acid isomers such as **D-KDGal** could be easily accessed.

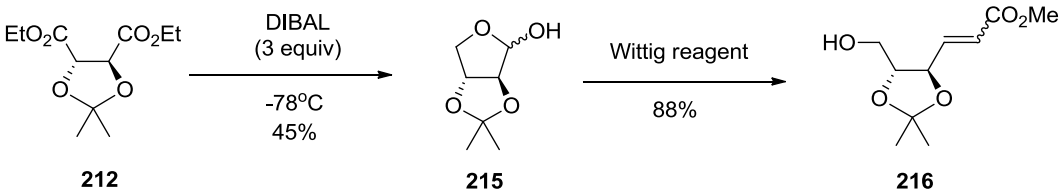
2.4 Synthesis of D-KDGal

When planning the synthesis of **D-KDGal**, D-tartaric acid **217** was identified as a commercially available C4-synthon with the correct stereochemistry to synthesise **D-KDGal**. Navarro had already shown that the acetonide protected diester of D-tartaric acid **212** could be reduced to diol **213**, followed by a one-pot oxidation and (*Z*)-selective Wittig reaction to give γ-hydroxyl mono-olefination product **214** (Scheme 54).[29] Similarly, Seebach has shown that three molecular equivalents of DIBAL could be used to reduce **212** to lactol **215**, with a Wittig reaction then affording the γ-hydroxyl mono-olefination product **216** (Scheme 55).[30] More recently, Tomioka developed Seebach's strategy further by optimising the DIBAL reduction methodology and telescoping the reaction *via* addition of a deprotonated phosphonate ester.[31] The reaction scope was shown to enable a range of phosphonate esters to be used and had been scaled-up from 1 mmol to a 40 mmol scale with no loss in yield.

Scheme 54 *Navarro methodology for the synthesis of α,β-unsaturated ester* ***214***

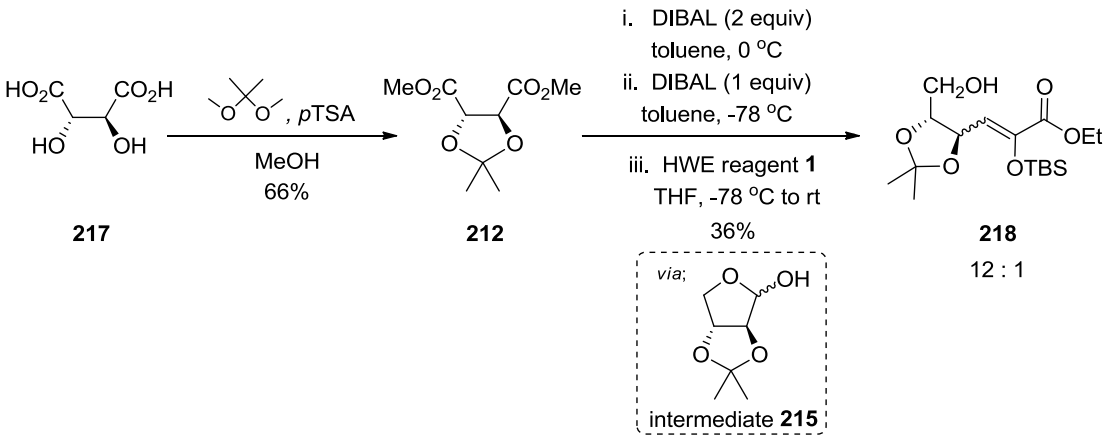


Scheme 55 *Seebach methodology for the synthesis of α,β-unsaturated ester* ***216***



Therefore, the Tomioka protocol was employed to react acetonide diester **212** with phosphonate ester **1**, successfully giving the desired product **218** in 36% yield over two steps (Scheme 56). The low yield was a little disappointing, however two reactions had been carried out in one pot, using cheap starting materials, with pure product being efficiently separated from over-reduced and di-olefinated side-products by silica gel column chromatography.

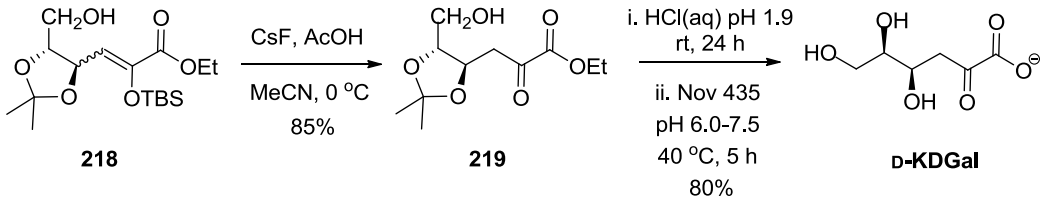
Scheme 56 *Synthesis of Silyl-Enol Ether* ***218***



O-silyl deprotection was then carried out using the same CsF/AcOH methodology that was used to successfully deprotect the **D-KDG** precursor **199**. Spectroscopic analysis of the crude product revealed that the deprotection had been successful, but surprisingly **219** was not a mixture of pyranose anomers as previously observed for **D-KDG** precursor **200**. A peak at δ 192 ppm in the ^{13}C NMR spectrum indicated that the free ketone was present,

and this was confirmed by the diastereotopic C(3)H^AH^B shifted to δ 3.1 ppm and 3.2 ppm because of their position α to a ketone functional group (downfield compared to the C(3)H_2 peaks of α/β-**200** found at δ 2.3-1.9 ppm). The acetonide deprotection and ester hydrolysis method developed for the synthesis of **D-KDG** was then carried out to afford **D-KDGal** in an overall yield of 17% over just four steps with $[\alpha]_D^{25}$ -5.5 (*c* 3.6, MeOH-H_2O 1:1)(lit.[32] $[\alpha]_D^{25}$ +7.3 (*c* 2.0, H_2O), with spectroscopic data matching that reported in the literature (Scheme 57).

Scheme 57 *Final steps for the synthesis of* ***D-KDGal***



218 **219** **D-KDGal**

2.5 Sugar Isomeric Composition in Solution

Unlike most small molecules, sugars do not exist in solution as one structure, but instead can occur as mixtures of as many as seven isomers at equilibrium. Attempts have been made to accurately predict the isomeric composition of sugars, using molecular mechanics to predict the free energies of isomers,[33-35] but these methods often fail to systematically predict sugar composition in aqueous solution. This is in part due to calculations modelling sugar molecules in the gas phase or in apolar solvents, rather than in aqueous solution. In aqueous solution, solvation of the sugar molecules plays a significant role in stabilising different isomeric forms, which is a factor that cannot easily be predicted. Therefore, to discover the isomeric composition of sugars in solution, there is still no substitute for experimental evidence. In the past many methods have been applied to this problem: polarimetry;[36] circular dichroism;[37] IR;[38] GLC;[39] and Raman spectroscopy.[40] By far the most regularly used technique has been ^1H, ^{13}C NMR spectroscopic analysis and in the case of phosphorylated sugars, ^{31}P NMR.[41-42] More recently, preparation of molecules containing ^{13}C labels and integrating ^{13}C NMR signals has proven to be a powerful technique for discovering the presence of minor isomers.[43]

The isomeric composition of **D-KDGal** and **D-KDG** in solution was determined using a combination of 1D and 2D ^1H and ^{13}C NMR experiments. The assignments of isomers for **D-KDGal** and **D-KDG** were confidently made for the pyranose and acyclic forms using their distinct coupling constants and chemical shifts respectively. However, the assignments of the furanose isomers could only be made tentatively using the previous

assignments of Thiem who synthesised **D-KDGal** and **D-KDG** and reported ^{1}H NMR spectra that closely matched our results.[44]

2.5.1 D-KDGal Isomeric Composition in Solution

The ^{1}H NMR spectrum for **D-KDGal** is complicated by overlapping resonances of the five isomers that are present (Figure 7). This could be deconvoluted by total correlation spectroscopy (TOCSY), where a resonance of a nucleus is correlated with chemical shifts of all nuclei of the same kind that are within its spin system. This technique allowed identification of which resonances pertained to which isomer, even when many resonances were overlapping in the parent ^{1}H NMR spectrum (Figure 9). The coupling constants for H^{3ax} and H^{3eq} to H^{4} allowed the major constituent isomer to be identified as the α-pyranose isomer, since the large H^{3ax} to H^{4} coupling constant of 11.5 *Hz* must be due to an axial-axial interaction (Figure 8). For sugars, pyranose forms are generally more stable than their corresponding furanose and acyclic isomers, so it was unsurprising that only minor amounts of the β–furanose, α-furanose and acyclic forms were present (11%, 9% and 3% of the isomeric compostition respectively). Consideration of the structure of the α-pyranose isomer reveals why this isomer is favoured over the β-pyranose isomer (72% and 5% of the isomeric composition respectively). For the α-pyranose isomer, apart from the C(2)-OH, all of the ring substituents are in equatorial positions, minimising 1,3-diaxial steric repulsions. However, for the β-pyranose isomer, its lowest energy chair conformer has all three hydroxyl groups in unfavourable axial positions.

Figure 7 *Isomeric composition of **D-KDGal** in D_2O at 25 °C*

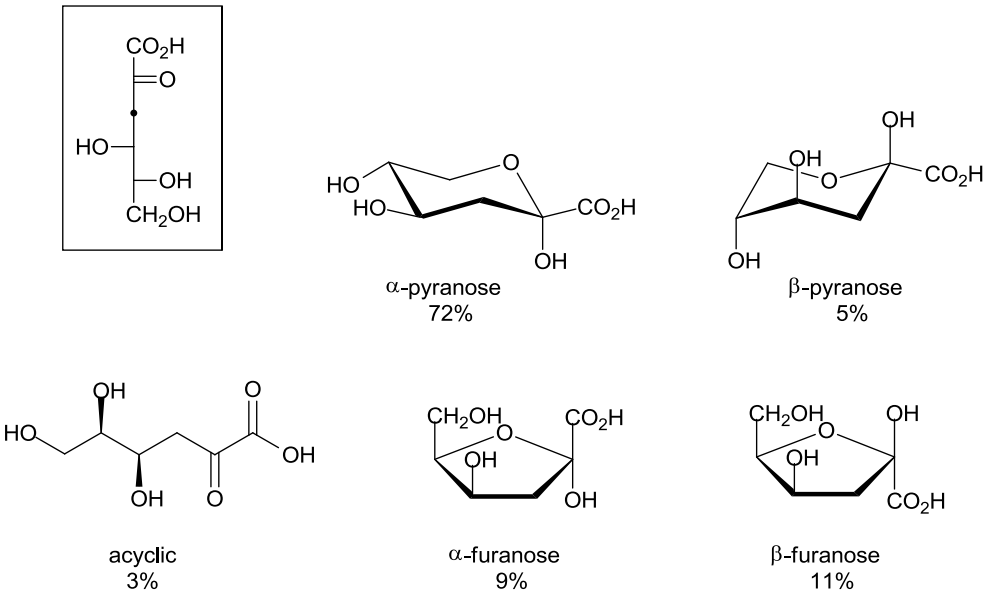


Figure 8 *^{1}H NMR spectrum of* ***D-KDGal*** *with 1.6 ppm – 2.2 ppm region showing key coupling constant values for major α-pyranose isomer*

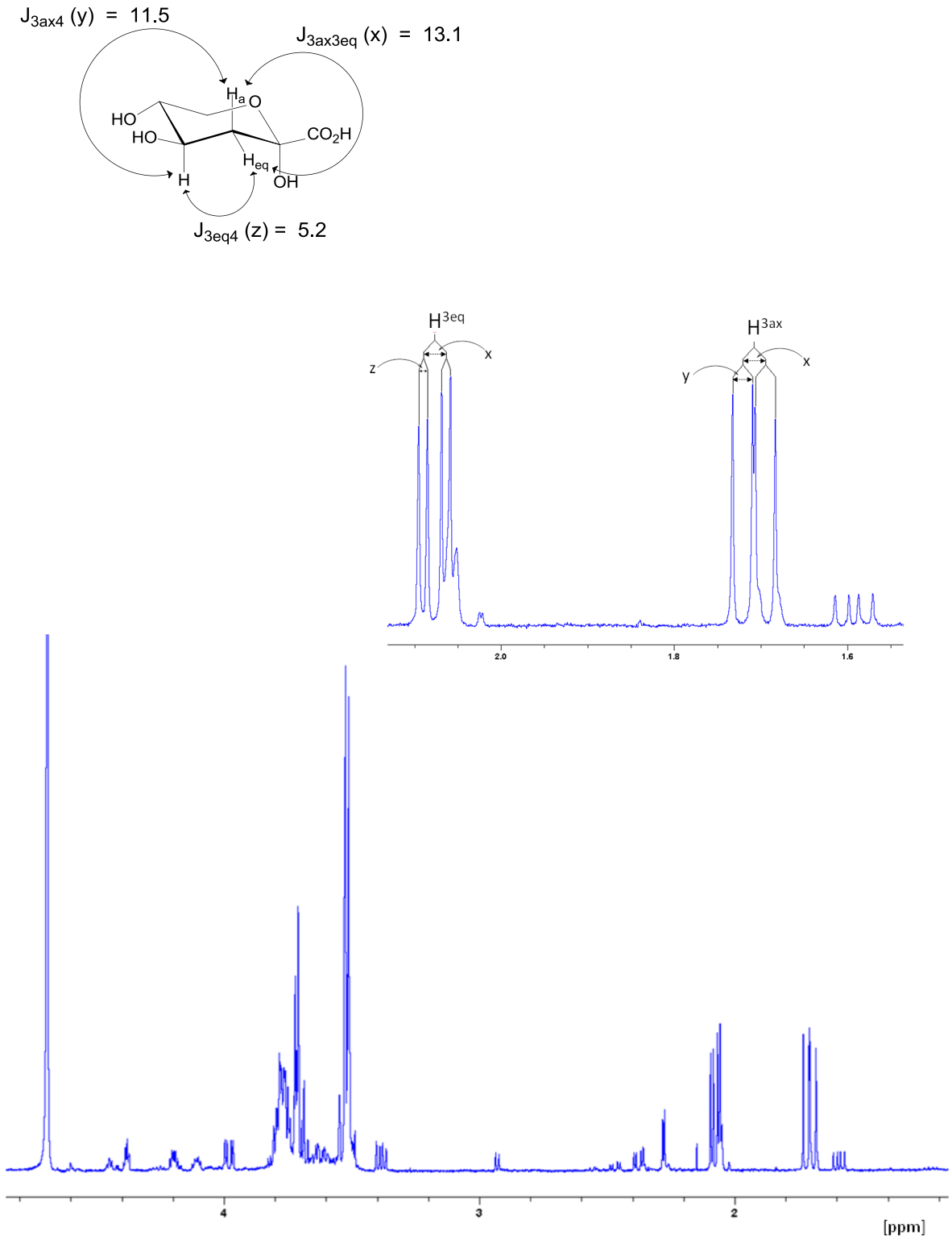


Figure 9 *^{1}H NMR spectrum of* ***D-KDGal*** *in D_2O*

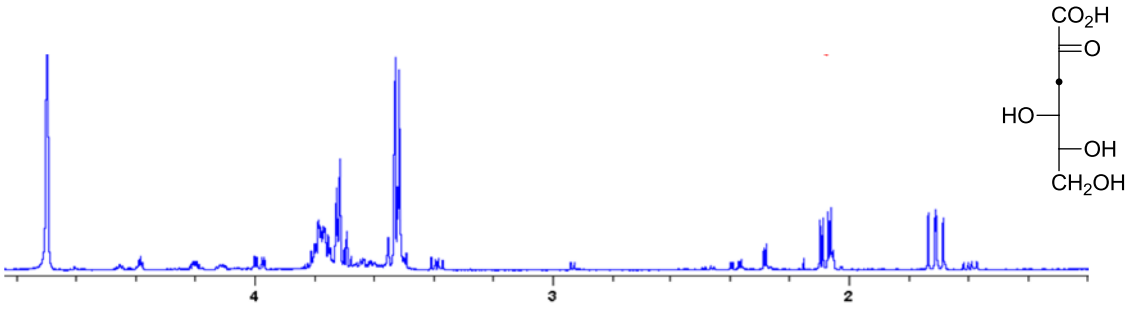
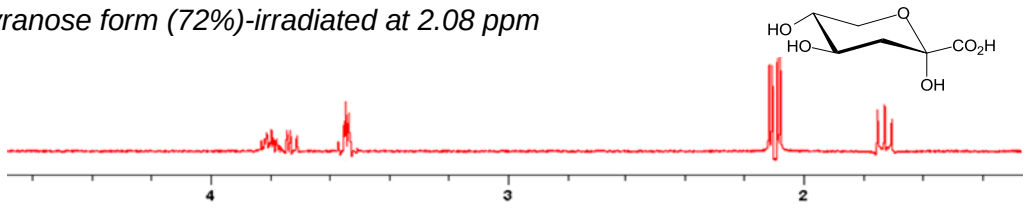
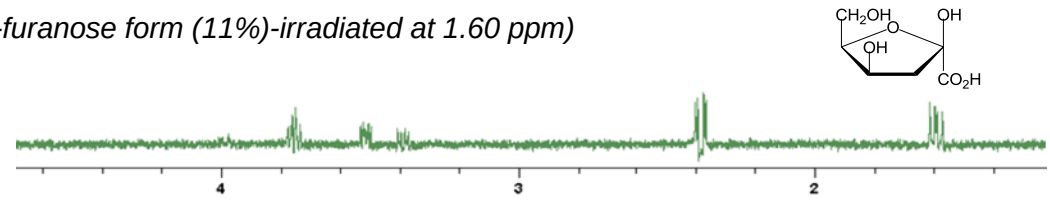


Figure 10 *TOCSY ^{1}H NMR spectra of* ***D-KDGal*** *(percentage of isomeric composition in parantheses)*

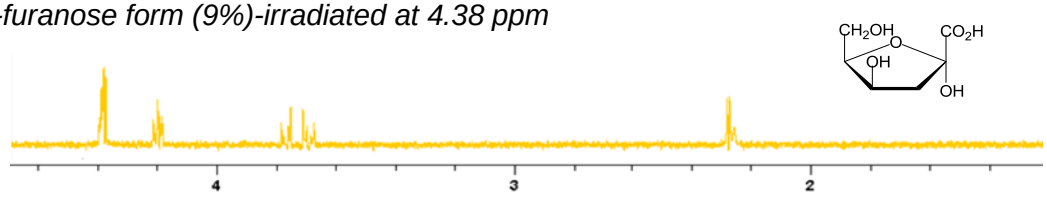
i.*α-pyranose form (72%)-irradiated at 2.08 ppm*



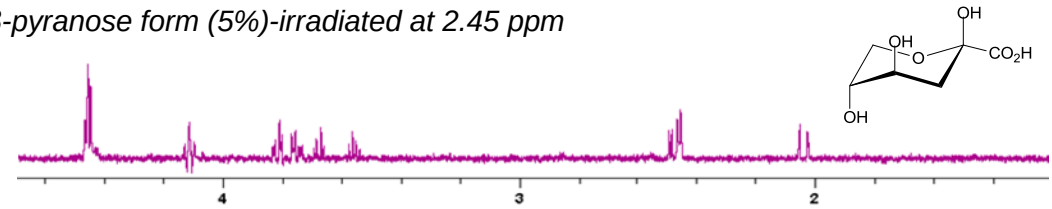
ii. *β-furanose form (11%)-irradiated at 1.60 ppm)*



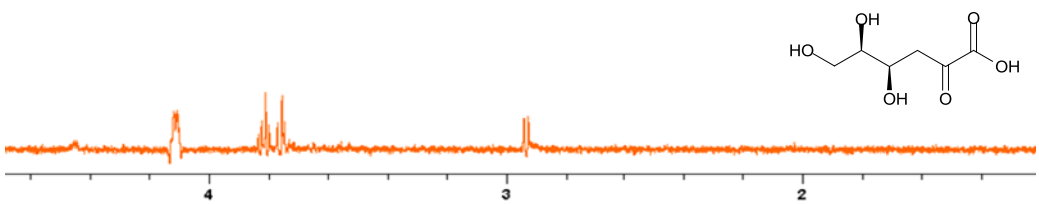
iii. *α-furanose form (9%)-irradiated at 4.38 ppm*



iv. *β-pyranose form (5%)-irradiated at 2.45 ppm*



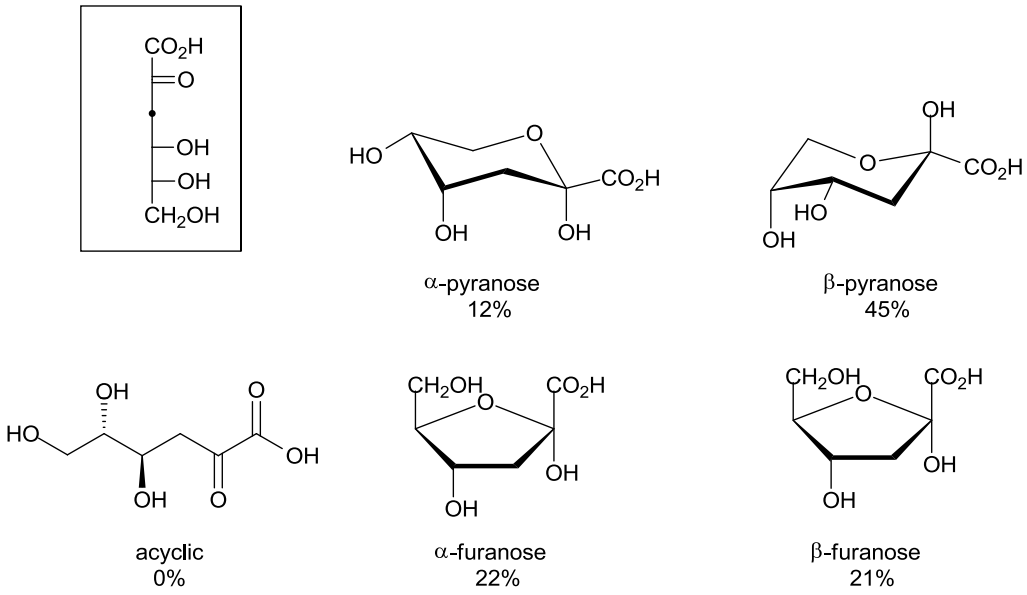
v. *acyclic form (3%)-irradiated at 4.11 ppm*



2.5.2 Isomeric composition of D-KDG in solution

Again, TOCSY and COSY NMR analysis allowed the even more complicated ^{1}H NMR spectrum of **D-KDG** to be deconvoluted (Figure 12 and 13). The main isomer, making up 45% of the isomeric mixture, was shown to be the β-pyranose ring form due to a large coupling constant for H^{3ax} to H^{4} of 12.2 *Hz* as a result of H^{4} positioned in the axial position. The composition of isomers for **D-KDG** was found to be significantly different to that of **D-KDGal**. The β-pyranose isomer of **D-KDG** was not nearly as relatively stable as the α-pyranose isomer of **D-KDGal**, which is due to the **D-KDG** β-pyranose isomer having its C(4) hydroxyl group in its axial position. It is also interesting to note that there was no acyclic isomer present for **D-KDG** (cf. **D-KDGal** (3%)).

Figure 11 *Isomeric composition of **D-KDG** in D_2O at 25 °C*



It is interesting to compare these results with those obtained previously for D-sorbose (Figrue 14i) and D-fructose (Figure 14ii), [45-46] which are ketose 'equivalents' of **D-KDGal** and **D-KDG** respectively (Figure 14 (i) and (ii)). It is striking that D-sorbose has the highest level of α-pyranose isomer (98%), just like **D-KDGal**, whilst D-fructose has its β-pyranose isomer as the major constituent (65%), but with substantial percentages of the furanose isomers analogous to **D-KDG** (32% in total).

Figure 12 *^{1}H NMR spectrum of* ***D-KDG*** *in D_2O*

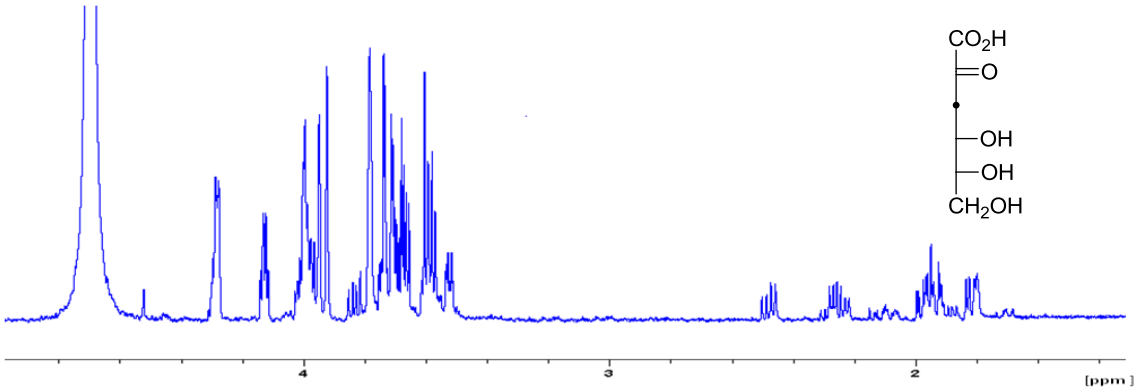
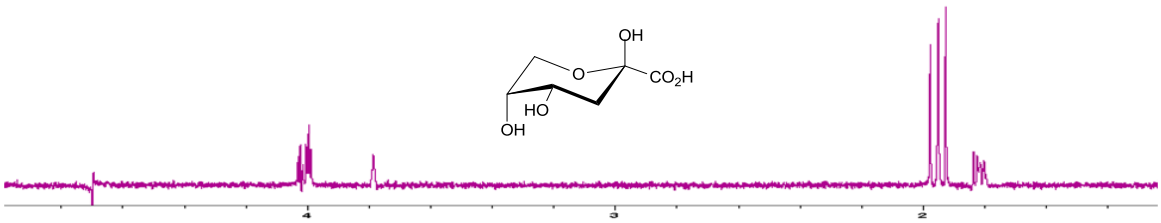
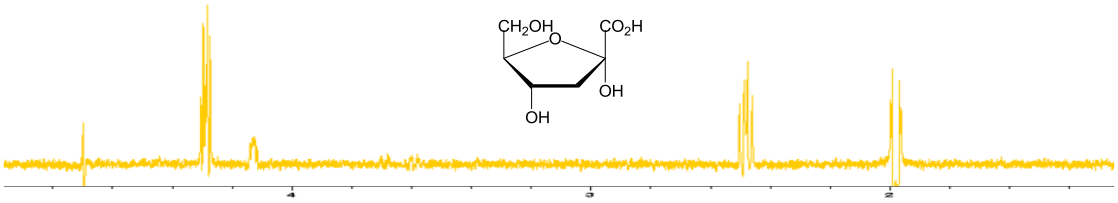


Figure 13 *TOCSY ^{1}H NMR spectra of* ***D-KDG*** *(percentage of isomeric composition in parantheses)*

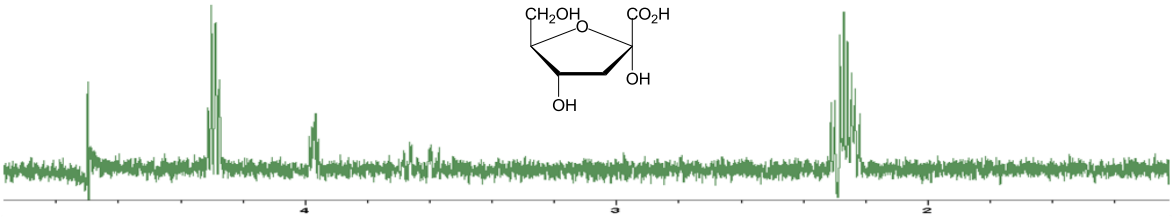
i. *β-pyranose form (45%)-irradiated at 1.81 ppm*



ii. *α-furanose form (22%)-irradiated at 2.49 ppm*



iii. *β-furanose form (21%)-irradiated at 2.26 ppm*



iv. *α-pyranose form (12%)-irradiated at 2.08 ppm*

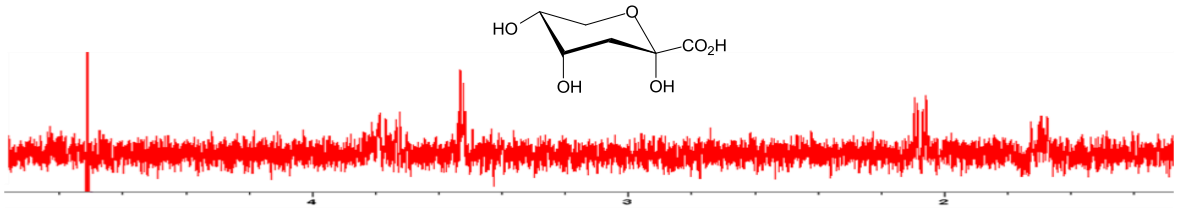
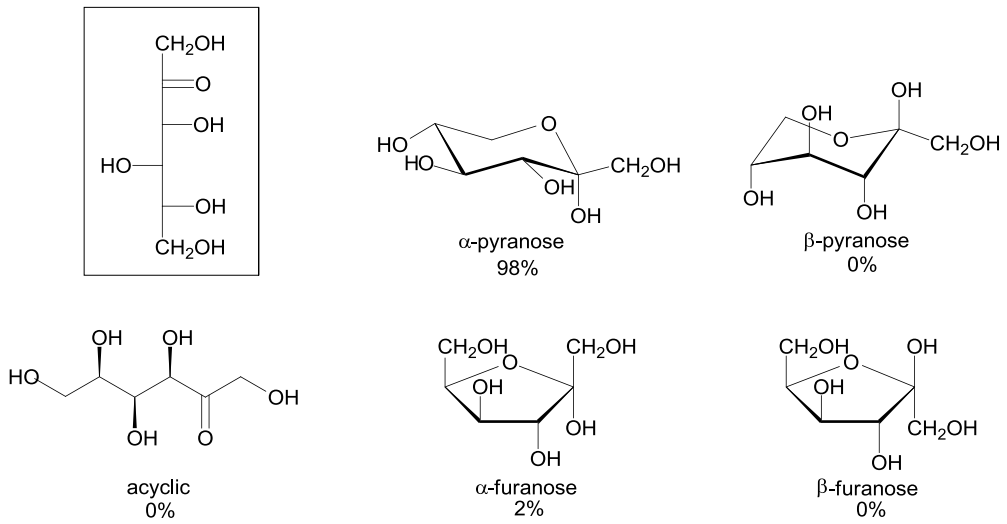
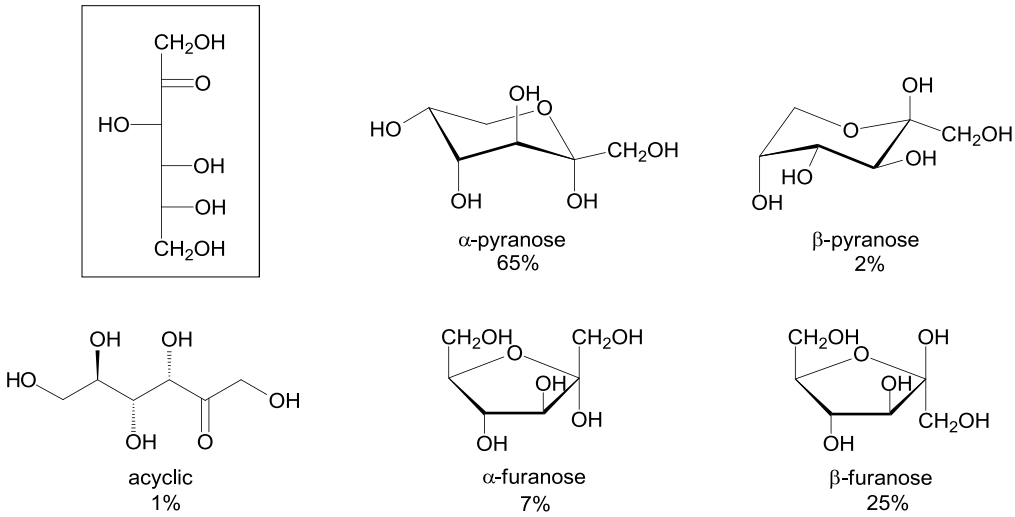


Figure 14 Isomeric c*omposition of (i) D-sorbose and (ii) D-fructose in solution*

(i) *D-sorbose at 27 °C*[45]



(ii) *D-fructose at 31 °C*[46]

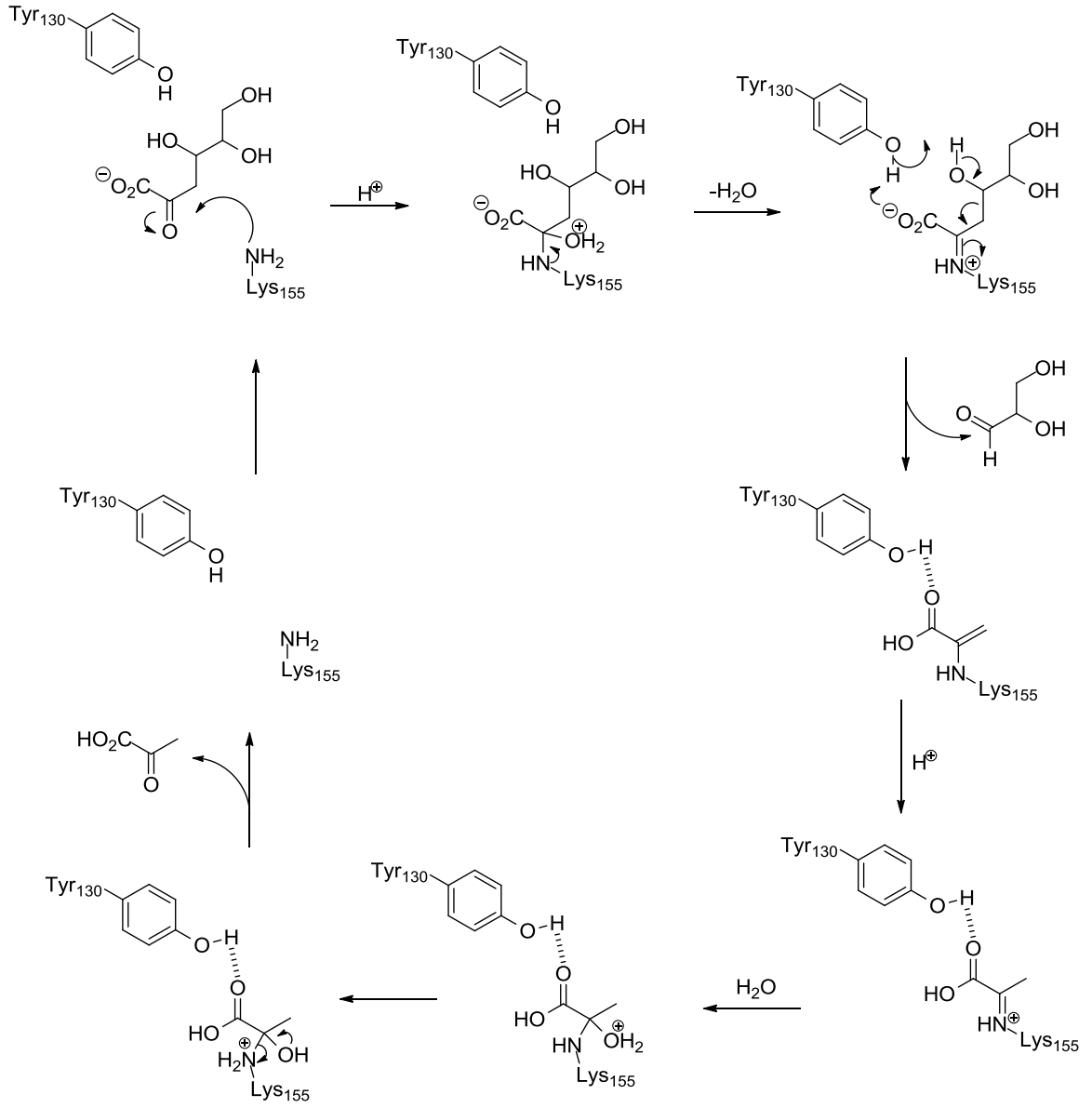


2.6 Variation of the Isomeric Composition of D-KDG and D-KDGal with Temperature

It has been shown that **D-KDG** and **D-KDGal** are efficiently catabolised by KDG aldolase. The mechanism of action for this aldolase, which has been substantiated by X-ray structures of substrates and intermediates bound to its active site, involves reaction of a lysine residue with the acylic isomer of the sugar to form a Schiff base intermediate, before a tyrosine mediated *retro*-aldol reaction occurs to catalyse reversible cleavage of the carbon-carbon bond (Scheme 58).[47] Since the enzyme acts on the sugar in its acyclic form this prompts the question; how is it possible that **D-KDG** and **D-KDGal** are such good

substrates for this enzyme despite having a small percentage of their acyclic isomers present in aqueous solution?

Scheme 58 *Mechanism of KDG aldolase from S. solfataricus*[47]



It is known that temperature, solvent and time have an effect on the isomeric composition of sugars in solution.[36] Since *S. solfataricus* is a hyperthermophilic archaeon that grows optimally at 80 °C, it was decided to determine how the isomer composition of sugars can change as a function of temperature, with the aim of determining whether more of the acyclic form predominates at higher temperatures. A sample of each sugar was heated to the desired temperature and then left to equilibrate for one hour before acquiring a quantitative ^{1}H NMR spectrum, the isomer ratio being determined by the relative peak integrals of the different isomeric forms.

The results proved most striking for **D-KDG** (Figure 15). Increasing the temperature from 25 °C to 60 °C gave a definite and gradual increase in the percentage of acyclic isomer present, from 0% at room temperature to 16% at 60 °C, with a concomitant decrease in the major β-pyranose isomer from 45% to 22%. The same trend could be seen for **D-KDGal** (Scheme 16); with the percentage of acyclic isomer increasing from 3% at room temperature to 16% at 60 °C, and the major α-pyranose isomer decreasing from 72% to 48%. The respective quantities of both α- and β-furanose isomers also increase with temperature for both **D-KDG** and **D-KDGal**, but to a lesser extent than that observed for the acyclic isomers.

Figure 15 *Isomeric composition as a function of temperature for* ***D-KDG***

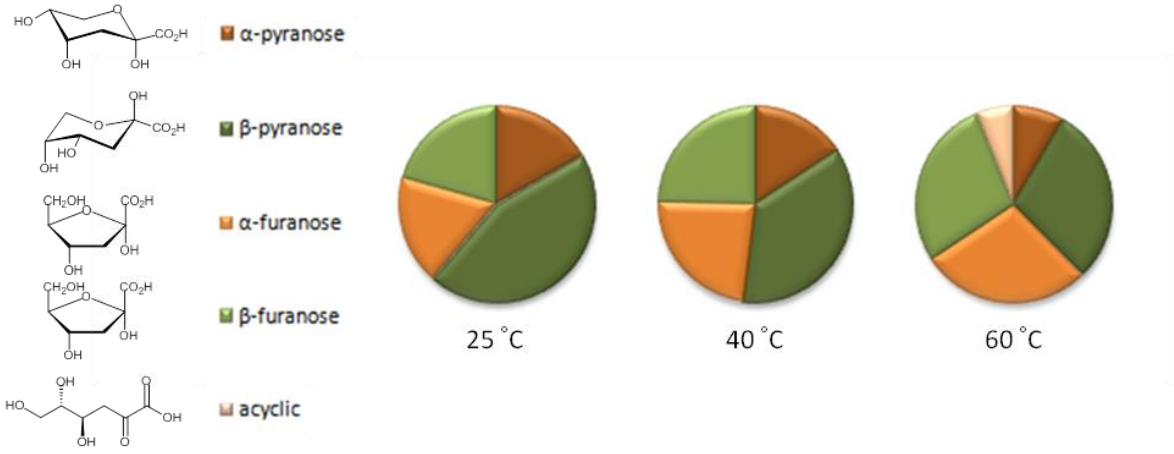
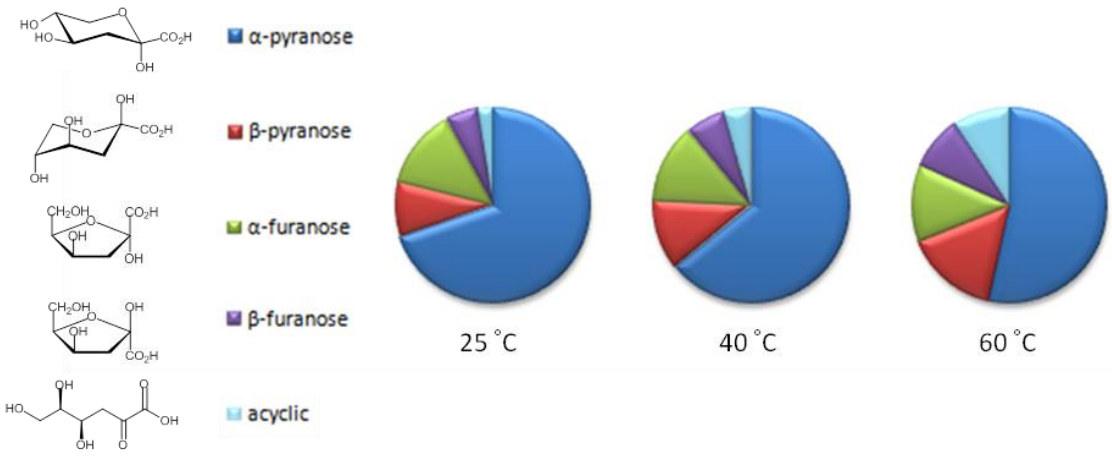


Figure 16 *Isomeric composition as a function of temperature for* ***D-KDGal***



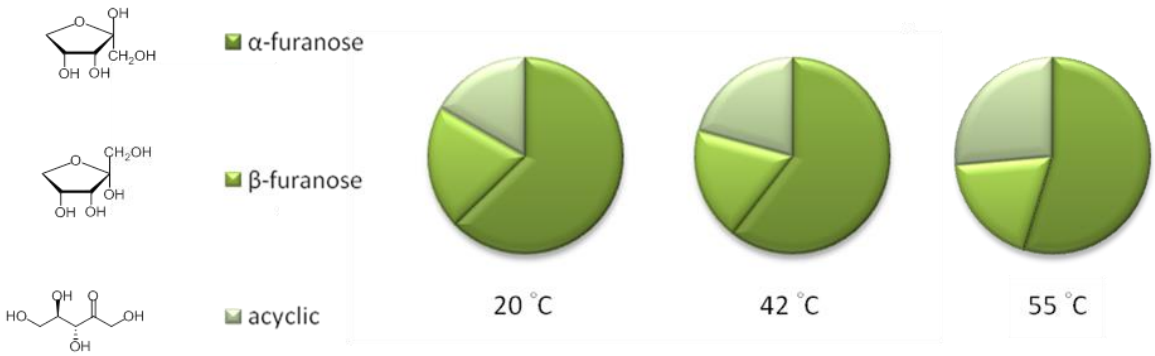
The increase in percentage of acyclic and furanose isomers with temperature has not been reported previously for ulosonic acids, but it is interesting to note that the trend is

paralleled for aldoses and ketoses.[48] For example, Lichtenthaler has reported that the isomeric ratios of α-D-glucopyranosyl-D-fructoses, were affected by temperature to varying extents in D_2O, with a general increase in the percentage of furanose isomers observed as temperature increased.[42] In addition, Serriani has published work on the effect of temperature on the isomeric composition of D-2-pentuloses (Figure 17 i and ii).[49] He observed a similar trend for increased formation of acyclic isomer as observed for **D-KDG** and **D-KDGal**. The trend to form a greater amount of acyclic isomer at higher temperature would be expected from considerations of entropy, whereby the acyclic form has greater conformational mobility and therefore becomes more energetically stable than the cyclic forms with increasing temperature.[36] However, some studies have shown that the enthalpy term can also be significant: with the acyclic and furanose isomers having higher enthalpy and increasing in proportion with increasing temperature.[50] Furthermore, the ring closure formation of hemiacetals is exothermic and therefore the equilibrium towards the cyclised products is favoured at lower temperature.[36]

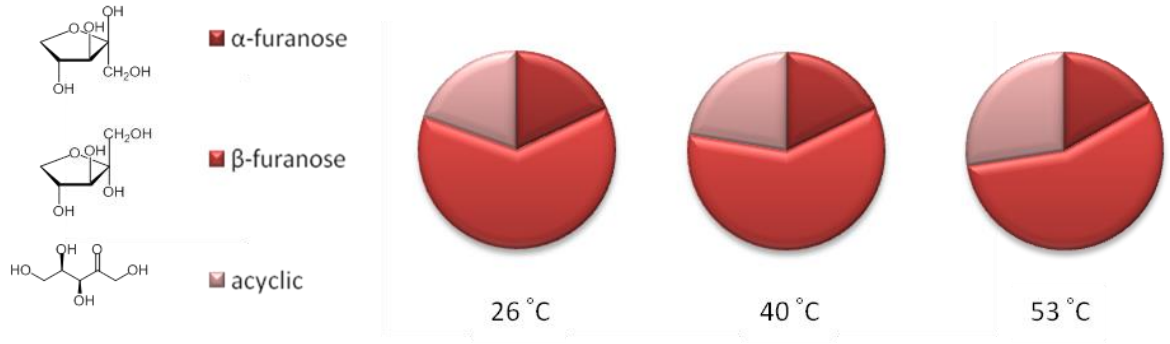
The increased acyclic percentage composition of these 3-deoxy-hex-2-ulosonic acids at raised temperatures goes some way to explaining how KDG aldolase can efficiently catabolise these sugars. However, whilst conducting the variable temperature ^{1}H NMR experiments it was also demonstrated that the rate of mutarotation of these 2-keto-3-deoxy-ulosonic acids was relatively rapid at room temperature in D_2O, since the percentage isomeric composition five minutes after raising the solution temperature from 25 °C to 40 °C, matched the isomeric composition after one hour. Therefore, consumption of the small amount of open-chair isomer of **D-KDG** and **D-KDGal** present will perturb the isomer equilibrium, resulting in mutarotation to afford more open-chain form, thus allowing the *retro*-aldol reaction to go to completion.

Figure 17 *Results reported by Serianni on the isomeric composition of D-2-pentuloses as a function of temperature*[a]

(i) D-Ribulose



(ii) D-Xylulose



[a]Conditions: 0.3M pentulose, 15% (v/v) D_2O, 50 mM acetate buffer, pH 4.0.

However, it must also be noted that although the mechanism of KDG aldolase has been reported to proceed *via* an acyclic intermediate,[47] initial coordination of these sugars to the protein in one or other cyclic forms followed by a subsequent protein induced ring opening event cannot be discounted. Indeed Kragl has concluded that this type of initial coordination event occurs for the sialic acid aldolase catalysed *retro*-aldol reaction,[51] citing NMR results from Brossmer as further evidence.[52] Activation of this kind by KDG aldolase would further explain how the enzyme can efficiently catabolise these 3-deoxy-hex-2-ulosonic acids efficiently.

2.7 Conclusion

D-KDG and **D-KDGal** have been synthesised *via* a concise four step route from naturally occurring sugar substrates. These routes make use of HWE reactions between the anion of ethyl 2-((*tert*-butyldimethylsilyl)oxy)-2-(dimethoxy-phosphoryl) acetate **1** with enantiopure sugar-derived aldehydes to afford silyl-enol ethers that could be globally deprotected to give the target 2-keto-3-deoxy-ulosonic acids in high purity. This synthetic methodology will be of interest to the wider biochemistry community since it should provide efficient routes to this class of α-keto sugars that are ubiquitous metabolites for archaea, bacteria and eukarya.

These C6-sugars are now being used as substrates for the directed evolution of a stereochemically promiscuous aldolase from *S. solfataricus* to develop mutant aldolases with high diastereoselectivity for the aldol reaction of D-glyceraldehyde **6** and pyruvate **7** to exclusively afford either **D-KDG** or **D-KDGal** (Section 1.4). This project has already begun in collaboration with members of the CER at Bath where libraries of 10^4–10^5 aldolase

mutants have been generated by ep-PCR. The initial validation of these libraries has been carried out by transforming plasmids into a pyruvate kinase deficient *E. coli* auxotroph and their growth on minimal media supplemented with pyruvate revealed a number of extant colonies after incubation for 72 hours. The growth of the mutant *E.coli* auxotroph colonies on pyruvate supplemented minimal media demonstrates that these *E. coli* auxotroph libraries are viable and therefore the directed evolution project screening for *retro*-aldolase activity towards **D-KDG** and **D-KDGal** can now begin. Daughter plates will be prepared with one plate supplemented with **D-KDG** and the other with **D-KDGal**, with comparison studies identifying mutant colonies that are diastereoselective in the *retro*-aldol direction. The principle of microscopic reversibility then predicts that a mutant KDG aldolase that catalyses a diastereoselective *retro*-aldol reaction, will also catalyse a stereoselective aldol condensation reaction. This will be confirmed by identifying and purifying the aldolase gene sequence of diastereoselective mutant colonies and screening the resultant mutant aldolases for reaction in the aldol condensation direction of D-glyceraldehyde **6** with pyruvate **7**. If the high-throughput screening methodology is successful for this directed evolution towards **D-KDG** and **D-KDGal**, the same methodology will then be used to screen for mutants with increased substrate scope, in particular to discover mutants that accept aldose substrates with increased chain length or reduced hydroxyl substitution patterns.

2.7 References

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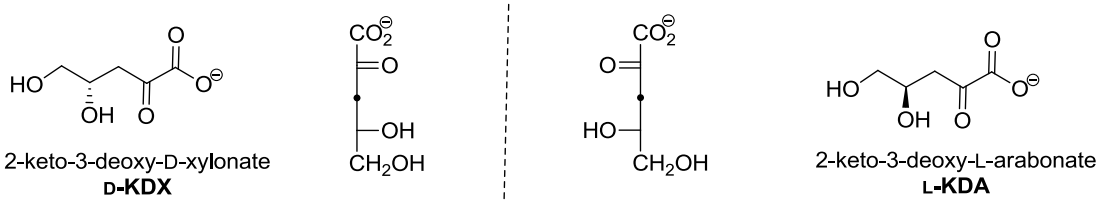
Chapter 3

3.1 Introduction

As described in Section 1.5, previous studies into the metabolism of D-xylose **8** and L-arabinose **9** by the archaeon *S. solfataricus* had shown that some of the enzymes involved in their metabolism were promiscuous towards these sugars and their metabolites. In order to confirm this hypothesis, efficient stereoselective syntheses of **D-KDX** and **L-KDA** (Figure 18) are now described, and the kinetic parameters of KDG aldolase (isolated from *S. solfataricus*) for the *retro*-aldol cleavage of these two enantiomers determined.[1]

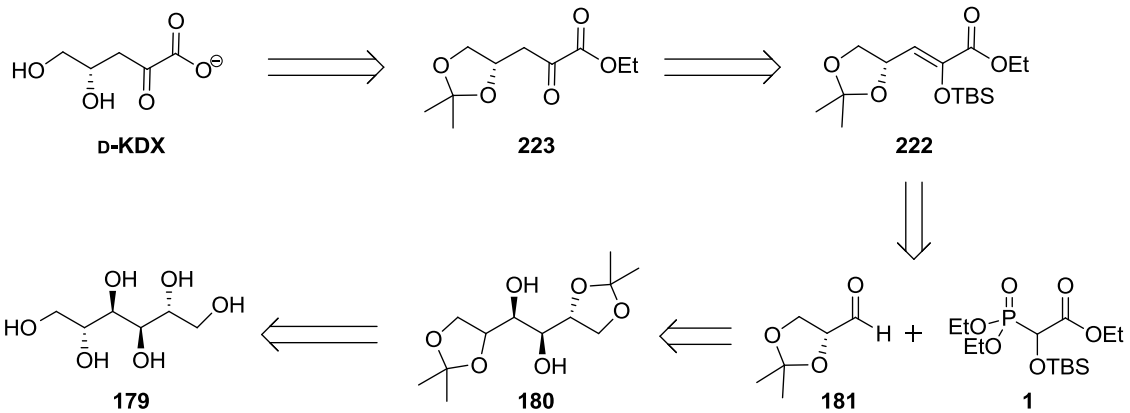
Figure 18 *Structures of the enantiomers* ***D-KDX*** *and* ***L-KDA***



3.2 Synthesis of D-KDX

It was decided to apply the methodology developed in the previous chapter for the synthesis of **D-KDG** and **D-KDGal** to the synthesis of the C5-sugar **D-KDX** (Scheme 59). Therefore, D-glyceraldehyde acetonide **181** would be synthesised and reacted with the HWE phosphonate substrate **1** to furnish the protected C5-sugar **222**. This would then be globally deprotected to furnish **D-KDX** in just five steps from D-mannitol **179**.

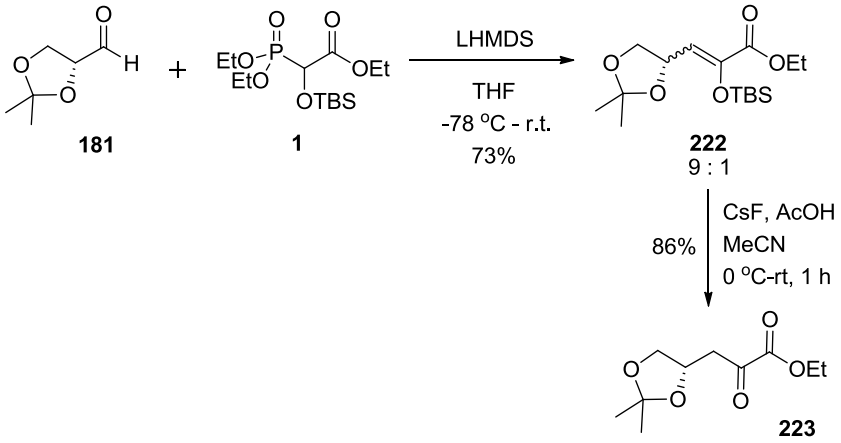
Scheme 59 *HWE retrosynthetic strategy for the synthesis of* ***D-KDX***



3.2.1 Horner-Wadsworth-Emmons reaction for the synthesis of D-KDX-acetonide ethyl ester 223

Phosphonate ester **1** was deprotonated using LHMDS at -78 °C and then reacted with D-glyceraldehyde acetonide **181** to give an inconsequential mixture of (*E*)- and (*Z*)-geometrical isomers of silyl-enol ester **222** in good yield. Desilylation using CsF then gave α-keto-ester **223** in high yield, with no further purification required (Scheme 60).

Scheme 60 *HWE step*



3.2.2 Deprotection Attempts

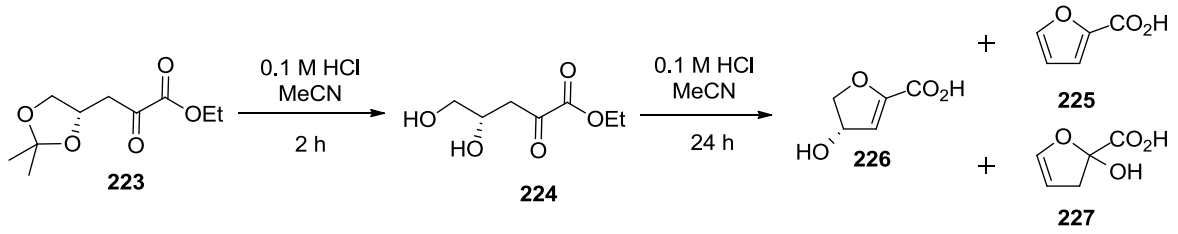
All that remained for the successful synthesis of **D-KDX** was the removal of the acetonide protecting group and hydrolysis of the ester functionality. Ordinarily, these transformations are straightforward reactions that have been achieved using a host of different reagents and reaction conditions. However, **D-KDX** proved to be an extremely challenging and elusive target that was found to be unstable under many of the conditions trialed.

Attempted base- and acid-promoted deprotection of α-keto ester 223

Initially a series of acidic deprotection protocols were trialed, with the expectation that the acetonide deprotection step would proceed with concomitant ester hydrolysis. For example, after stirring α-keto ester **223** in 0.1 M $HCl_{(aq)}$/acetonitrile for two hours, the crude reaction product was concentrated and partitioned between water and ethyl acetate. Analysis of the organic extracts showed that ester **224** had been formed in good yield after acetonide deprotection. Encouraged by this early success the reaction was repeated with a longer reaction time of 24 hours. However, analysis of the organic extracts revealed no organic products. Furthermore, when the aqueous phase was concentrated and analysed in D_2O by ^1H NMR spectroscopy, a complex mixture of products was observed. There were numerous peaks between δ 5.0-7.5 ppm at chemical shifts

corresponding to alkenyl protons, suggesting that unwanted dehydration reactions had occurred. This was not wholly unexpected, since it has previously been observed that **D-KDX** is prone to dehydration under acidic conditions.[2] It was tentatively suggested, from analysis of the ^{1}H and ^{13}C NMR spectra, that these dehydration products were furan-2-carboxylic acid **225** and mono-dehydration products **226** and **227** (Scheme 61). Indeed this conclusion was later justified by further decomposition studies on an authentic sample of **D-KDX**, *vide infra*.

Scheme 61 *Attempted acid- and base-mediated deprotection of α-keto ester **223***



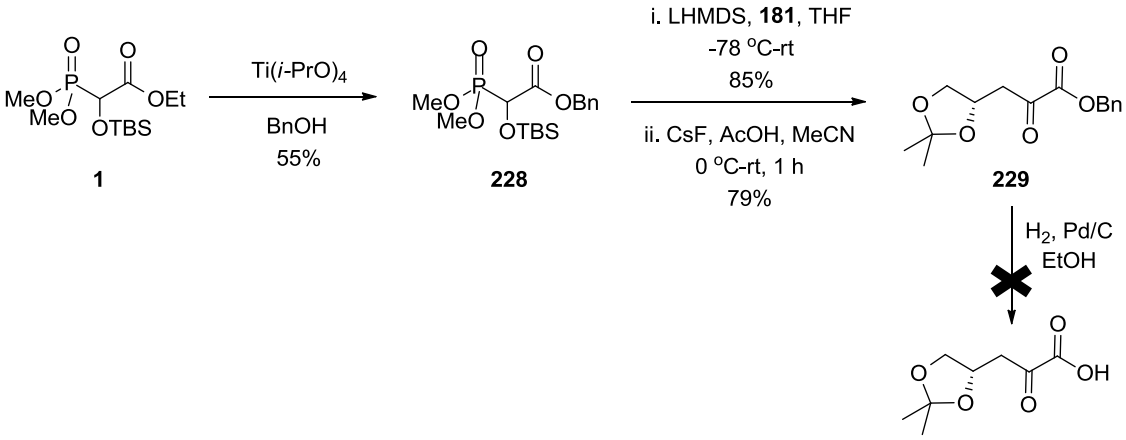
Given the problems encountered with this deprotection reaction, subsequent trials were conducted in deuterated solvents with levels of substrate conversion and product compositions monitored by in-process ^{1}H NMR spectroscopic analysis. For acidic conditions, a range of organic and mineral acids (*p*-TSA, Ambelite IR 120+, H_2SO_4, HCl) were trialed, which resulted in elimination reactions occurring simultaneously with acetonide deprotection and saponification. Unfortunately, a series of basic ester hydrolysis methods were also unfruitful, giving complex mixtures of products that did not contain any **D-KDX**.

Attempted hydrogenolytic deprotection

It was well known that benzyl esters could be reduced under hydrogenation conditions to their corresponding carboxylic acid derivatives, which in the proposed synthesis of **D-KDX** would obviate the problematic acidic and basic hydrolysis methods. Therefore, phosphonate ester **1** was transesterified with benzyl alcohol to give benzyl ester **228**. Elaboration of **228**, using the HWE/desilylation methodology already described, successfully gave α-keto benzyl ester **229** in good yield (Scheme 62). Unfortunately, hydrogenolysis of **229** to remove the benzyl substituent proved problematic. Stirring an ethanolic mixture of **229** with 10% palladium on carbon catalyst under an atmosphere of H_2 for one hour resulted in all of the starting material being consumed to afford polar baseline products. After work-up, analysis of the crude product by ^{1}H NMR clearly showed that there were no aromatic proton peaks present, confirming the successful removal of the benzyl group. However, the NMR spectroscopic also data indicated the formation of a complex mixture of products that, despite having peaks in the correct

regions, did not match the ^{1}H or ^{13}C NMR spectra of **D-KDX**.

Scheme 62 Synthesis of b*enzyl ester **229** and its attempted hydrogenolytic deprotection*



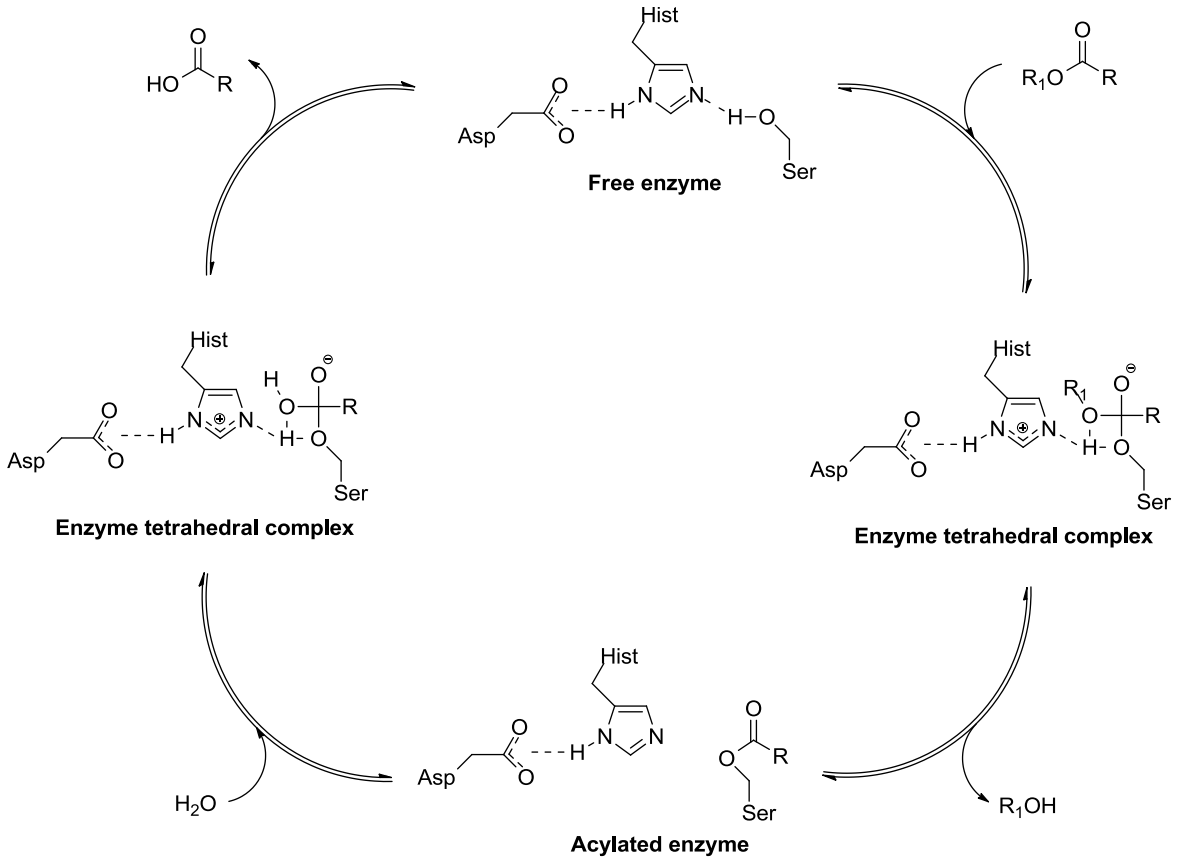
3.2.3 Lipase-Catalysed Hydrolysis of α-Keto Ester 223

After the failure of both acid- and base-catalysed hydrolysis and hydrogenolytic methods, an enzyme-catalysed hydrolysis method was investigated. It was hoped that by trialing a range of commercially available lipases, an enzyme could be found that would efficiently hydrolyse the ethyl ester of **223** to afford **D-KDX**, under very mild conditions. Lipases are a special type of esterase that catalyse the hydrolysis of esters that are active at lipid/water interfaces, thereby allowing them to catalyse the hydrolysis of water-insoluble substrates. There is great potential for the use of lipases for organic synthesis, since they are environmentally friendly, recyclable biocatalysts that operate under mild reaction conditions. Lipases make up a significant part of commercial enzyme sales as part of an industry that is valued at over $2 billion/year.[3] They are chiefly used in the detergent industry, paper manufacturing and food production. In organic synthesis they have demonstrated efficacy as catalysts for the selective hydrolysis of esters,[4] regioselective esterification and transesterification,[5] and even catalyse some unexpected reactions such as the aldol and Michael reactions.[6]

Lipase B from *Candida antarctica* (CalB) is a commercially available lipase that has received particular interest in recent years. It was originally isolated from a strain of basidomycetous yeast found in Antarctica, as its name suggests. *C. antarctica* produces two lipases, named A and B. Lipase A is a calcium dependant enzyme that is highly thermostable and active towards triglycerides, but has low activity for the hydrolysis of simple esters.[7] Contrastingly, CalB requires no co-factors, has lower thermostability and boasts a broad specificity profile towards esters, amides and thiols.[8] CalB is composed of 317 amino acids with a molecular weight of 33 kD and a pI of 6.0. It is a robust protein with optimum activity at pH 7.0, but is stable in the range of pH 3.5-9.5. Depending on the

pH of the reaction media it denatures in the range of 50-60 °C and is available as a pure protein, or immobilised onto a solid support as Novozym 435 (Nov 435) that is comprised of recombinant CalB supported on a macroporous acrylic resin. Immobilized CalB exhibits similar activity to that of the free recombinant enzyme, but with an increased thermal stability that enables it to be used at temperatures as high as 60-80 °C.[9] The tertiary structure of CalB has an α/β hydrolase fold and its mode of action, like other lipases, is comparable to that of a serine protease.[10] The active site is comprised of a flat hydrophobic region with a Ser-His-Asp "catalytic triad" playing a key role in the catalytic cycle (Scheme 63). Initially, the histamine residue helps to remove a proton from the serine alcohol, allowing the serine oxygen anion to attack the ester carbonyl bond. The tetrahedral intermediate is stabilised by hydrogen bonding to the negatively charged oxygen atom as it sits in an 'oxyanion' hole.[11] Expulsion of the alcohol group leaves an acylated enzyme intermediate, which is then attacked by a water molecule to give a new enzyme tetrahedral intermediate stabilised by hydrogen bonding to the histamine residue. Re-formation of the carbonyl bond gives the acid product and restores the catalytically active serine nucleophile of the lipase to its free state.

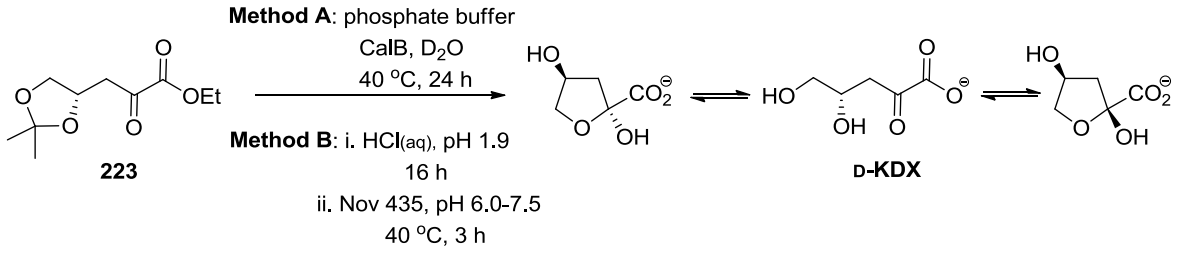
Scheme 63 *Catalytic cycle of Candida Antarctica lipase B*



Initially CalB was added to **223** in a solution of toluene-d_8 1:20 D_2O (v/v) buffered with potassium phosphate to pH 7.5 (Scheme 64). After 24 hours, in-process ^{1}H NMR spectroscopic analysis revealed the presence of ethanol and acetone, the absence of starting material and formation of **D-KDX** with only minor amounts of elimination by-products. It was apparent that as the ester group of **223** was hydrolysed to afford its corresponding acid, the resulting drop in pH from 7.5 to 1.0 was also sufficient to result in concomitant acetonide deprotection to afford **D-KDX**.

Next, Nov 435 was used in place of CalB to allow for ease of removal of the enzyme by filtration, with analysis revealing a crude product composition that was identical to the previous trial using soluble CalB. Although the enzyme was successful in achieving both transformations in one pot on a trial scale, it was thought that the low reaction pH might cause the enzyme to denature and lose activity on a larger scale. Therefore, the method was further developed into a one pot, two step procedure suitable for scale-up. Firstly, **223** was stirred for 16 hours in a dilute hydrochloric acid solution (pH 1.9). After that time, in-process ^{1}H NMR showed complete removal of the acetonide protecting group to afford diol **224**, with no elimination by-products having been formed. The reaction mixture was then neutralised (pH 7.0) with 1.0 M NaOH$_{(aq)}$ and warmed to 40 °C. Following the addition of Nov 435, the pH was maintained between 6.0-7.5 *via* dropwise addition of 0.1 M NaOH$_{(aq)}$. The reaction was monitored by in-process ^{1}H NMR spectroscopy that revealed clean hydrolysis after just three hours, with no elimination by-products present.

Scheme 64 *Successful two-step deprotection and hydrolysis of α-keto ester **223** to afford **D-KDX***



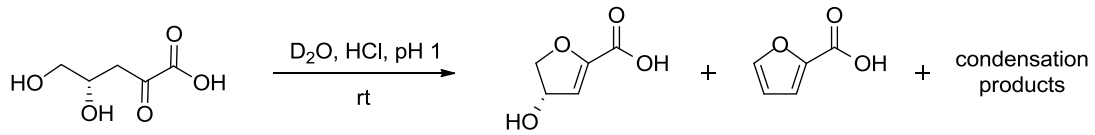
Attention was then turned towards the purification of **D-KDX**, since **D-KDX** of >98% purity was required for subsequent kinetic studies using KDG aldolase. Earlier attempts to purify **D-KDX** and **L-KDA** had not been successful, presumably due to the sugars' propensity to eliminate and polymerise under acidic conditions.[12] Initially, purification by ion-exchange chromatography using Dowex 1x8 resin in its acidic form and eluting with water/formic acid 0.05-1.0 M was trialed. HPLC analysis of the column fractions showed that pure product came off at eluent concentrations of 0.5-0.6 M formic acid. However,

concentration of these fractions gave impure oils that were contaminated with the elimination products previously observed. Elimination occurred despite addition of excess water to the column fractions in an attempt to azeotrope off the formic acid. After a meticulous search of the literature, it was found that 2-keto-3-deoxy-D-*manno*-octulosonic acid **16** had been chromatographed on silica gel TLC plates using the very polar eluent system of CH_2Cl_2-MeOH-H_2O, 5:5:1.[13] It was very pleasing to find that purification of crude **D-KDX** by silica gel chromatography using this solvent system afforded a sample of pure product, without the formation of elimination products upon concentration of the fractions *in vacuo*.

3.2.4 Impurities arising from acid- and base-catalysed decomposition of D-KDX

In order to more fully understand why ester hydrolysis had proven unsuccessful using Brønsted acids and bases, the stability of **D-KDX** under acidic conditions was investigated. **D-KDX** was stirred at room temperature in a D_2O solution carefully acidified with conc. HCl to pH 1 and monitored at regular intervals by ^{1}H NMR spectroscopic analysis (Scheme 65). In-process analysis after 14 hours showed that most of the **D-KDX** had decomposed, with the main constituent of the mixture being the mono-dehydration product **226**. After 48 hours, all of the **D-KDX** had been consumed, with the major constituent of the product mixture still being the mono-dehydration product **226**. However, a smaller quantity of furan-2-carboxylic acid **225** was present, as well as multiple products with proton resonances in the range of 1.5-4.0 ppm, which are likely to be due to condensation products arising from competing intermolecular reactions between **D-KDX**, **226** and **225**.

Scheme 65 *Products arising from acid treatment of* ***D-KDX***



D-KDX **226** **225**

When **D-KDX** was treated with LiOH in D_2O (0.2 M solution), it went on to form a complex mixture of products within 1.5 hours. The structures of these products could not be fully assigned, although it could be concluded that **D-KDX** was definitely unstable under these basic aqueous conditions, perhaps decomposing *via* base-catalysed *retro*-aldol reactions. The ^{1}H NMR spectra from these reactions were compared to the earlier acid- and base-catalysed acetonide deprotection and ester hydrolysis trials on α-keto ester **223**. There was a close correlation between the ^{1}H NMR spectra of both sets of experiments, which confirms that the instability of **D-KDX** under these acidic and basic reaction conditions was the reason for their failure to furnish **D-KDX**.

3.3 Synthesis of L-KDA

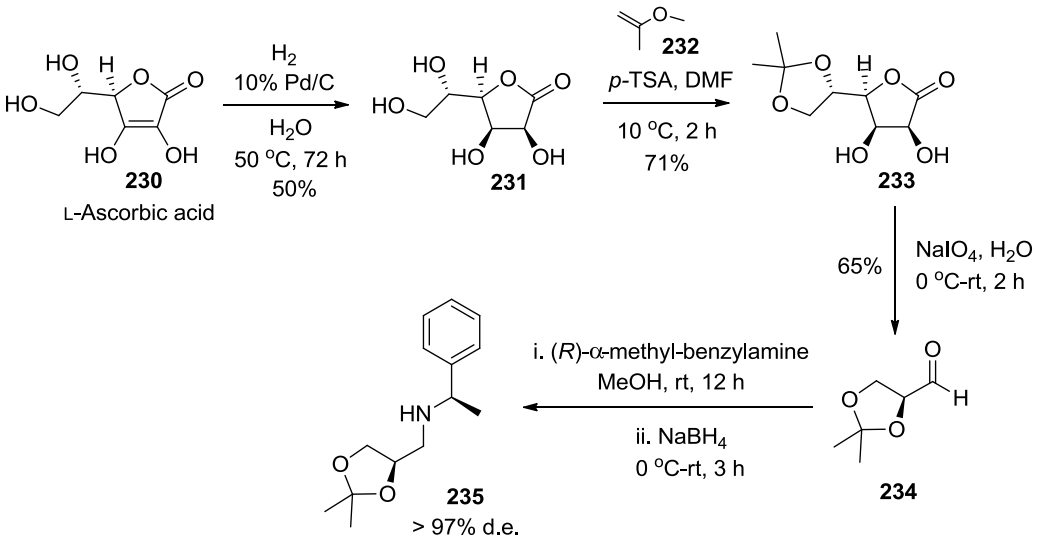
The HWE method developed for the synthesis of **D-KDX** was then applied to the synthesis of its enantiomer **L-KDA**. The correct configuration for **L-KDA** could be achieved using L-glyceraldehyde acetonide **234** as a substrate for the HWE reaction.

3.3.1 Synthesis of L-glyceraldehyde acetonide 234

L-Glyceraldehyde acetonide **234** was not commercially available and so it was necessary to synthesise it from a suitable chiral pool starting material. The literature synthesis of **223** in three steps from L-ascorbic acid **230** was chosen for its efficiency and the ready availability of cheap starting materials and reagents.[14]

L-Ascorbic acid **230** was first hydrogenated in an autoclave, using 150 psi of H_2 pressure and a catalytic quantity of 10% Pd/C. After recrystallisation from EtOAc/MeOH, lactone **231** was isolated as a white crystalline solid in good yield (Scheme 66). Lactone **231** was then treated with 1.3 equivalents of 2-methoxypropene **232** and a catalytic quantity of *p*-TSA in anhydrous DMF. This resulted in selective derivatisation of the terminal diol of **231**, rather than the internal diol fragment. The resultant acetonide **233** was allowed to react directly, without further purification, with sodium metaperiodate in water to give L-glyceraldehyde acetonide **234** in good yield with $[\alpha]_D^{20}$ -50 (*c* 0.5, $CDCl_3$) (lit.[15] $[\alpha]_D^{25}$ -54.9 (*c* 3.4, $CHCl_3$)). The percentage enantiomeric excess was confirmed by reductive amination with (*R*)-α-methylbenzylamine, whereupon analysis of the product by ^{1}H NMR spectroscopy revealed the presence of a single diastereomer (>97% d.e.).

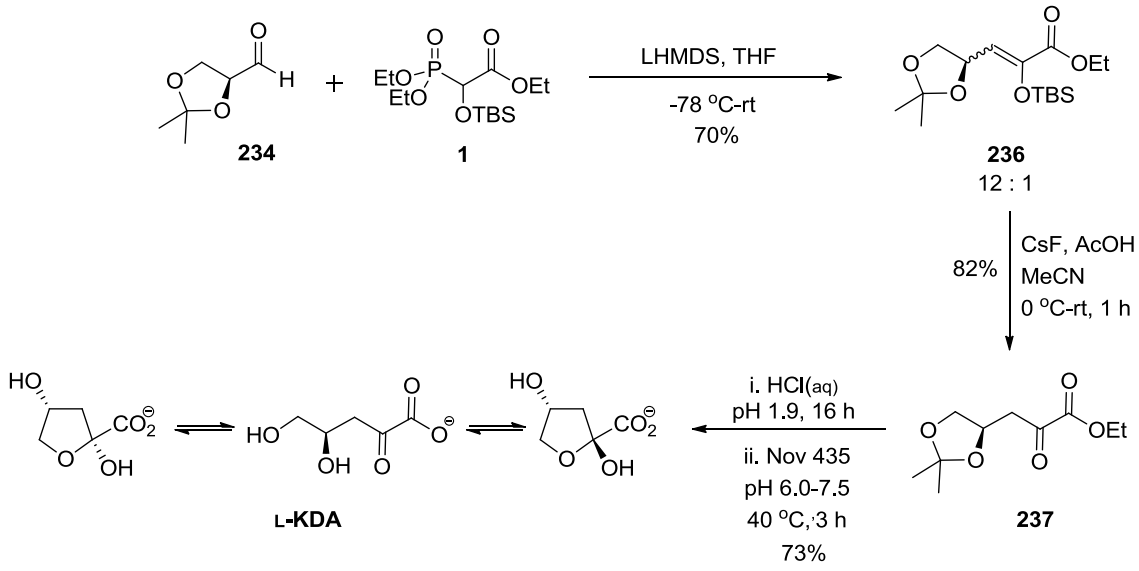
Scheme 66 *Synthesis of 2,3-O-isopropylidene-L-glyceraldehyde **234***



3.3.2 Synthesis and spectroscopic anaylsis of L-KDA

Aldehyde **234** was then used as a substrate to prepare **L-KDA** using the HWE/deprotection methodology previously developed for **D-KDX** (Scheme 67). **L-KDX** was synthesised and purified in good overall yield, with analytical data (MS, IR, ^{1}H and ^{13}C NMR spectra) identical to those previously obtained for **D-KDX**.

Scheme 67 *Synthesis of **L-KDA***



3.4 Isomeric Composition of D-KDX/L-KDA in Solution

The matching ^{1}H and ^{13}C NMR spectra of **D-KDX** and **L-KDA** revealed that these sugars exist as a mixture of three isomers in solution (Figure 19). The COSY spectrum of **D-KDX** made it possible to assign the peaks belonging to each isomer and confirmed that three rapidly interconverting isomers existed in solution (Figure 20). By comparing the ^{1}H integrals of the CH_2 peaks, which were clearly resolved in the 500 MHz NMR spectrum, it was possible to measure the relative proportions of these three isomers in D_2O. They existed in a ratio of 30:33:37 (acyclic:α-furanose:β-furanose) at 25 °C and pH 7. The acyclic isomer was clearly confirmed to be the α-keto form from the ^{13}C NMR spectrum, exhibiting a peak at 203 ppm due to the ketone carbon, with two peaks for α- and β-furanose isomers due to the anomeric carbons being present at 103.80 ppm and 108.85 ppm.

Figure 19 *Annotated ^{1}H NMR spectrum of* ***D-KDX*** *(25 °C, D_2O)*

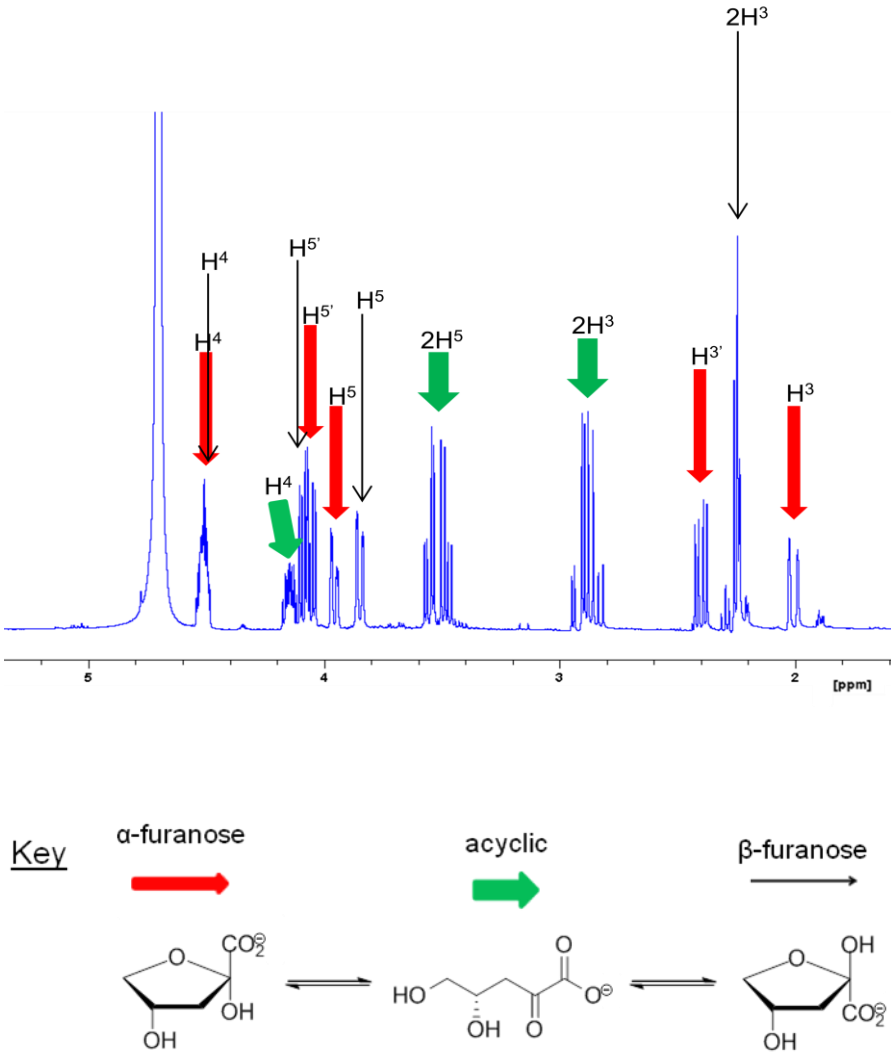
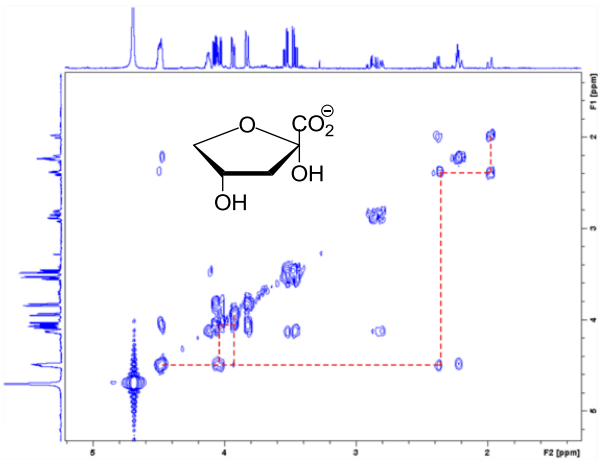
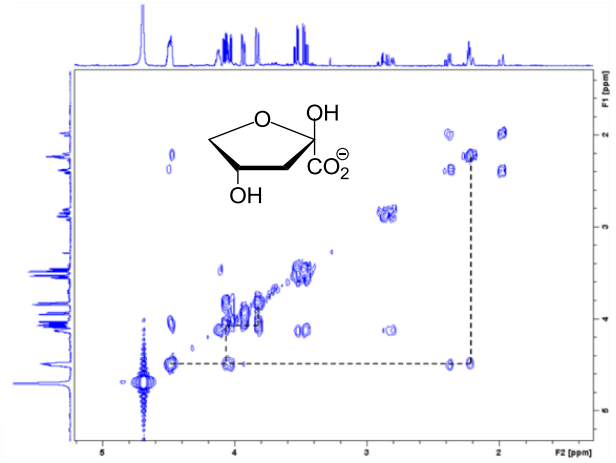


Figure 20 *COSY spectrum of* ***D-KDX*** *revealing interactions of resonances corresponding to the different isomeric forms: (i) α-furanose; (ii) β-furanose; and (iii) acyclic*

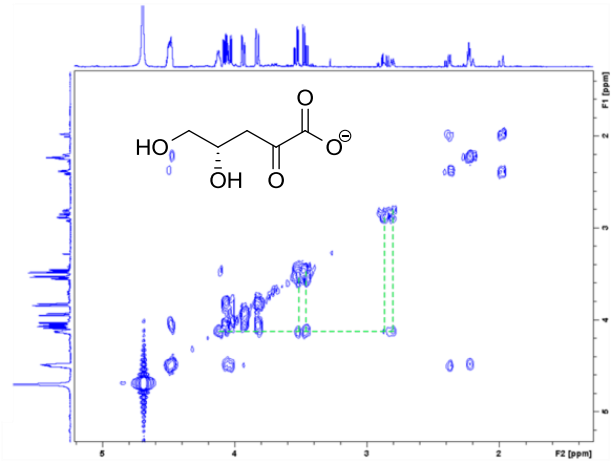
(i)



(ii)



(iii)

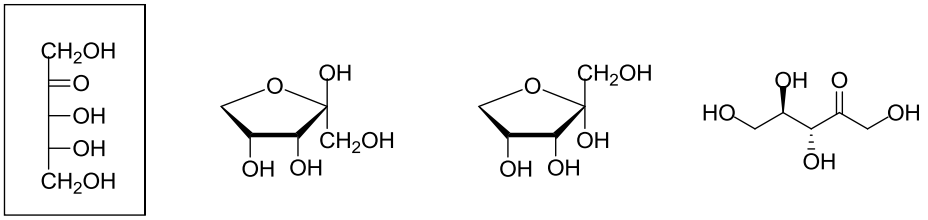


Resonances were assigned as the α- and β-furanose isomers by comparison with the chemical shifts previously observed for the α- and β-furanose isomers of **D-KDG**. Therefore, ^{1}H NMR spectroscopic resonances at 2.39 ppm and 1.97 ppm were assigned to the C(3)H_2 of the α-furanose isomer since they closely matched the C(3)H_2 α-furanose resonances of the sodium salt of **D-KDG** (2.48 ppm and 1.93 ppm). Conversely, the overlapping resonance centred at 2.23 ppm matched the chemical shift of the C(3)H_2 resonances of the β-furanose isomer of the sodium salt of **D-KDG** (2.29 ppm and 2.24 ppm). For **D-KDX** and **L-KDA** it appears that the carboxylate substituent must help stabilise the acyclic form, since its ethyl ester derivative **232** (Section 3.2.2) exhibited none of the acyclic isomer. The relatively large quantity of acyclic isomer also contrasts with the low quantities of acyclic isomer found for **D-KDG** (0%) and **D-KDGal** (3%) (Sections 2.6.1 and 2.6.2), which is probably due to the Thorpe-Ingold effect, where the extra ring substituent of the C6-sugars favours ring closure. However, **D-KDX** and **L-KDA** are not anomalous in having significant quantities of their acyclic isomers present in solution, as similar mixtures of cyclic and acyclic isomers have been seen previously for the 2-pentuloses D-ribulose **238** and D-xylulose **239**,[16-17] whilst for D-threose **240**,[18] the

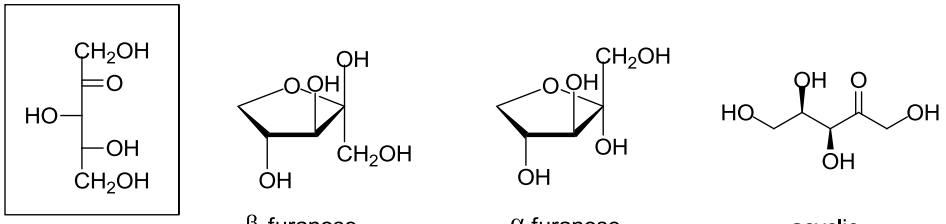
acyclic isomer was present mainly in its hydrated (*gem* diol) form (Figure 21). Indeed, for most aldose sugars it is known that in solution the more reactive aldehyde group of the acyclic form exists mostly in its hydrated form, but for ketose sugars the keto form dominates with no substantial quantities of hydrate.[19-20]

Figure 21 *Isomeric compositions of (i)* D*-ribulose* ***238****,*[25] *(ii)* D*-xylulose* ***239***[16] *and (iii)* D*-threose* ***240***[18]

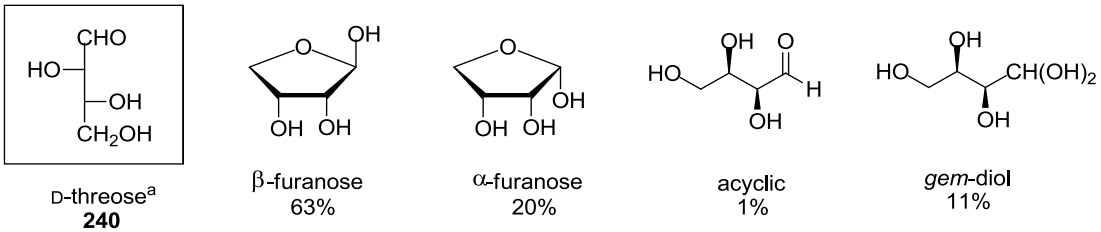
i.



ii.



iii.



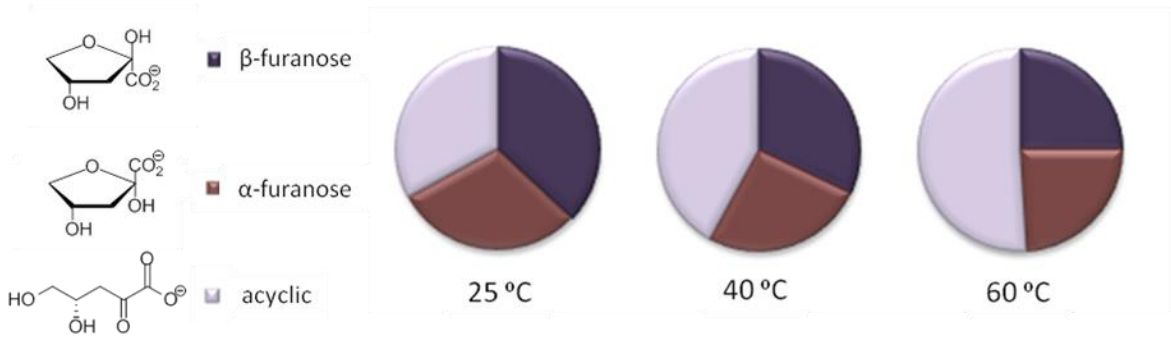
[a] composition at 20 °C; [b] composition at 26 °C.

3.5 Variation of the Isomeric Composition of D-KDX and L-KDA with Temperature

Increasing the temperature resulted in an increase in the percentage of acyclic keto isomers of **D-KDX/L-KDA**, as previously observed for the hexose sugars **D-KDG** and **D-**

KDGal (Section 2.7). Specifically, ^{1}H NMR spectroscopic analysis revealed that the percentage of acyclic isomer in D_2O increased from 33% at room temperature to 51% at 60 °C at the expense of both furanose isomeric forms (Figure 22).

Figure 22 *Isomeric composition as a function of temperature for* ***D-KDX/L-KDA***[a]



[a]Conditions: 0.35M sugar, D_2O, pH 7.0

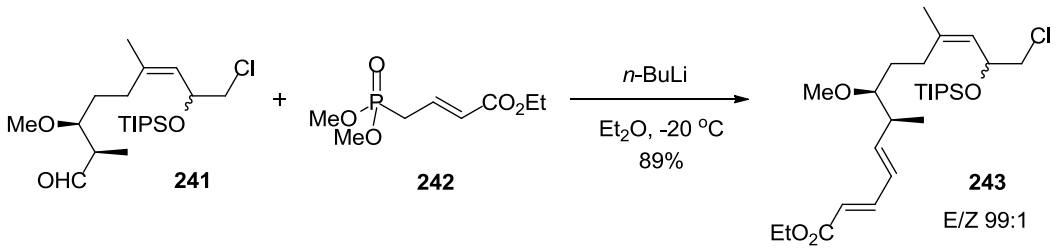
3.6 Confirmation of Enantiopurity of D-KDX and L-KDA

Samples of **D-KDX** and **L-KDA** which were prepared using the methods described above had analytical data that confirmed that their assigned structures were correct. However, since they were going to be used as enantiomerically pure substrates to assay KDG aldolase activity, it was crucial that their enantiopurity was also confirmed. Somewhat disturbingly, when their specific rotation was recorded for milligram quantities of each enantiomer in H_2O, the $[\alpha]_D$ readings were 0.01 and 0.00 for **D-KDX** and **L-KDA**, respectively! It was unclear whether this was due to the relatively low concentration (*c*) values used, or whether racemisation of the sugars' stereocentres had occurred during their syntheses.

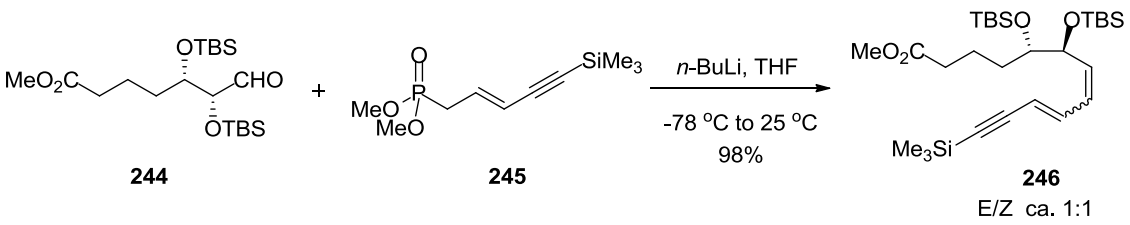
The synthetic route was re-examined in order to identify any steps that could potentially cause racemisation of the C(4) stereocentres provided by D-mannitol and L-ascorbic acid. Chiral derivatisation using enantiomerically pure (*R*)-α-methyl-benzylamine had confirmed that both the D- and L-glyceraldehyde acetonide intermediates were enantiomerically pure, so any racemisation event must have occurred after this point. LHMDS was used as the base for the HWE coupling reaction, which could potentially racemise the alpha stereocentre of each aldehyde. This was unlikely, however since HWE reactions had previously been carried out successfully without racemisation with aldehydes bearing an acidic α-stereocentre (Scheme 68).[21-23]

Scheme 68 *Literature examples of racemisation free HWE reactions with aldehydes containing acidic α-stereocentres*

(i)[21]



(ii)[23]



Further assurance was given by the observation that no epimerisation had been observed in the HWE reaction used for the syntheses of **D-KDG** and **D-KDGal**, which would have led to diastereomeric products that would have been detectable in the ^{1}H NMR spectra of their crude reaction products. For the three subsequent deprotection steps, the relatively mild conditions should not have led to any racemisation, especially as the stereocentre was no longer acidic. Indeed, the α-keto ester products **223** and **237** from CsF/AcOH-mediated deprotection had approximately equal and opposite optical rotation values [**223**-$[\alpha]_D^{30}$ +12 (*c* 1.25, CH_2Cl_2); **237**-$[\alpha]_D^{25}$ -8.9 (*c* 0.45, CH_2Cl_2)] confirming that the enantiopurity was likely to have been preserved throughout that reaction. However, it could not be discounted that racemisation of **D-KDX** and **L-KDA** had occurred in the subsequent acid-catalysed deprotection reaction *via* a reversible *retro*-aldol/aldol process.

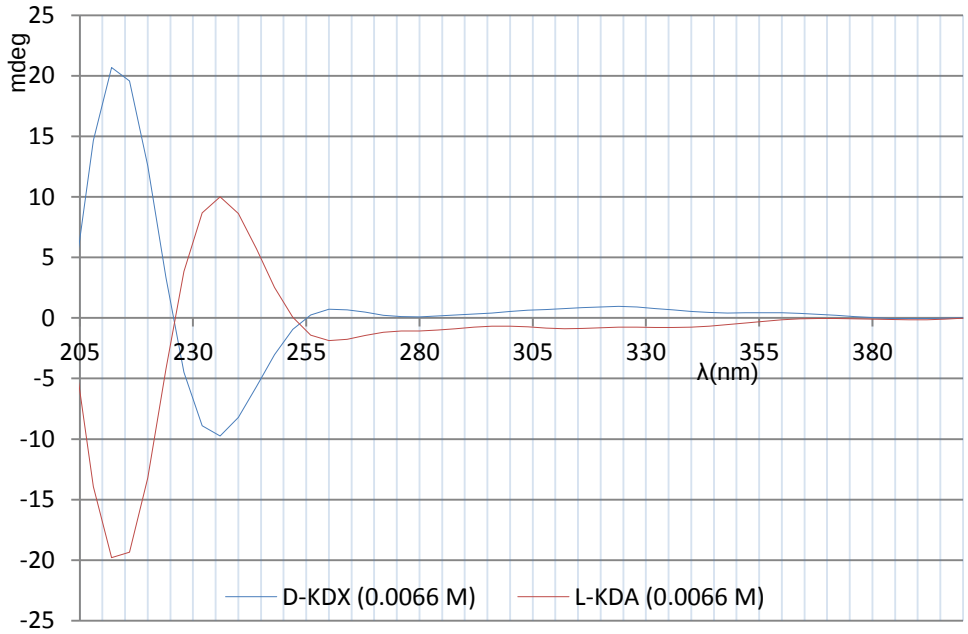
Once the pentose sugars had been re-synthesised on a larger scale, a screen of different solvent systems and the use of a higher concentration of sugar confirmed that **D-KDX** and **L-KDA** were optically active, giving optical rotation values of: $[\alpha]_D^{26}$ +13.5 (*c* 4.75, H_2O-MeOH, 1:1) for **D-KDX**; and $[\alpha]_D^{27}$ -13.3 (*c* 4.95, H_2O-MeOH, 1:1) for **L-KDA**. The fact that

these values were essentially equal and opposite in magnitude, strongly suggests that both **D-KDX** and **L-KDA** had been prepared in enantiopure form.

To gather further evidence for the enantiomeric purity of **D-KDX** and **L-KDA**, it was decided to record their optical rotatory dispersion (ORD) spectra (Figure 23). ORD spectra are dependent on the refractive index and also the absorption index of each isomer and normally give a distinctive Cotton effect close to the wavelength that a functional group absorbs at in the UV-vis spectrum. By far the most extensive studies of ORD spectra have been conducted on ketones, since the carbonyl chromophore normally absorbs at *ca.* 280-290 nm and the absorption is not usually strong enough to interfere with the ORD measurement. These ORD measurements have been widely used to probe structural features such as absolute configuration, the conformation of cyclic ketones in solution, absolute stereochemistry and the extent of ketal formation in solution.[24] The ORD spectra of α-keto carboxylic acids have also been studied by a number of groups with one or two bands in the range of 207-245 nm being observed and attributed to the n→π* absorption of different conformers.[25-26]

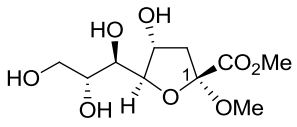
The key observation for the ORD spectra recorded for **D-KDX** and **L-KDA** were that the anomalous dispersion curves were equal and opposite in sign. When considered alongside the $[\alpha]_D$ readings that were equal and opposite in sign, this strongly implies that **D-KDX** and **L-KDA** had been successfully synthesised as pure enantiomers.

Figure 23 *ORD spectrum of* ***D-KDX*** *and* ***L-KDA***

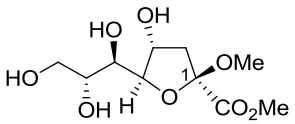


Looking at the dispersion curve of **L-KDA**, it can be seen that it exhibits a positive Cotton effect with a peak at 237 nm and a trough at 213 nm. The interpretation of this ORD curve is not trivial since the three isomeric forms of **L-KDA** present in solution could all be making contributions. However, previous work by the Redmond group, who managed to isolate pure anomers of the stable methylated of 3-deoxy-D-manno-oct-2-ulosonic acid, α-**247** and β-**247** (Figure 24),[27] were useful in helping with the interpretation. They found that α-**247** exhibited a positive Cotton effect at 210 nm, whilst β-**247** exhibited a mixed Cotton effect with a trough at 203 nm and a peak at 226 nm. Therefore, it can be speculated that the contribution of the signs of the ORD curves of the α-and β-furanose isomers of **L-KDA** at 215 nm are equal and opposite and therefore combine to cancel each other out, leaving a small positive contribution to the absorption spectrum from the β-furanose isomer at 226 nm. This combination of absorptions is then superimposed onto the contribution to the ORD spectrum from the acyclic isomer that contributes strongly towards the strong negative absorption at 215 nm.

Figure 24 *α- and β-isomers of the methylated 3-deoxy-D-manno-oct-2-ulosonic acid* ***247***



α-**247**

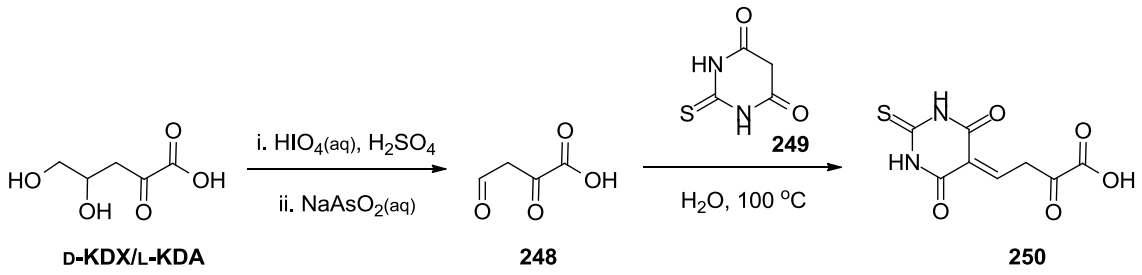


β-**247**

3.7 Kinetic Studies of KDG aldolase towards D-KDX and L-KDA

The kinetic parameters of KDG aldolase towards pure **D-KDX** and **L-KDA** for the *retro*-aldol cleavage reaction were then determined by running an assay over a range of sugar concentrations with all other variables kept constant. Two reactions were run for every concentration of sugar: one with KDG aldolase added at T_0; and a control that had no enzyme added. Each pair of reactions were conducted at 50 °C and stopped after ten minutes by the addition of aqueous trichloroacetic acid solution (10 µl, 12% w/v). The quantity of sugar starting material was then determined by a modified thiobarbituric acid (TBA, **249**) assay (Scheme 69), where the 1,2-diol C-C bond of **D-KDX** or **L-KDA** was cleaved by periodic acid, sodium arsenite added to quench the reaction, and then an aldol condensation reaction between TBA **249** and the aldehyde moiety of 2,4-dioxobutanoic acid **248** carried out to afford **250** that has a chromophore at 549 nm.[28-29]

Scheme 69 *Thiobarbituric acid assay used for determination of the activity of KDG aldolase towards* ***D-KDX*** *or* ***L-KDA***



The absorbance of **250** at 549 nm was recorded for each of the reactions, and the initial rate (*V*) was determined for each concentration by calculating the difference in the amount of **250** present with and without KDG aldolase. The KDG aldolase reaction is a single substrate reaction (Equation 1) and therefore the Michaelis-Menten equation (Equation 2) is obeyed.

Equation 1 *Single substrate enzyme reaction*

$$E + S \underset{k_{-1}}{\overset{k_1}{\rightleftharpoons}} ES \underset{k_{-2}}{\overset{k_2}{\rightleftharpoons}} E + P$$

Equation 2 *Michaelis-Menten equation*

$$V = (V_{max}.[S]) / (K_m+[S])$$

To determine the Michaelis-Menten constant (K_m) and the maximum rate (V_{max}), the kinetic assay results were used to make a Direct Linear plot (Equation 3),[30] which is derived from the Michaelis-Menten equation.

Equation 3 *Direct Linear plot equation*

$$V = ((V.K_m) / [S]) + V$$

$$(\; y = mx + c \;)$$

Using equation 3, two points were plotted for each sugar concentration:

When $K_m = 0$ $V_{max} = V$

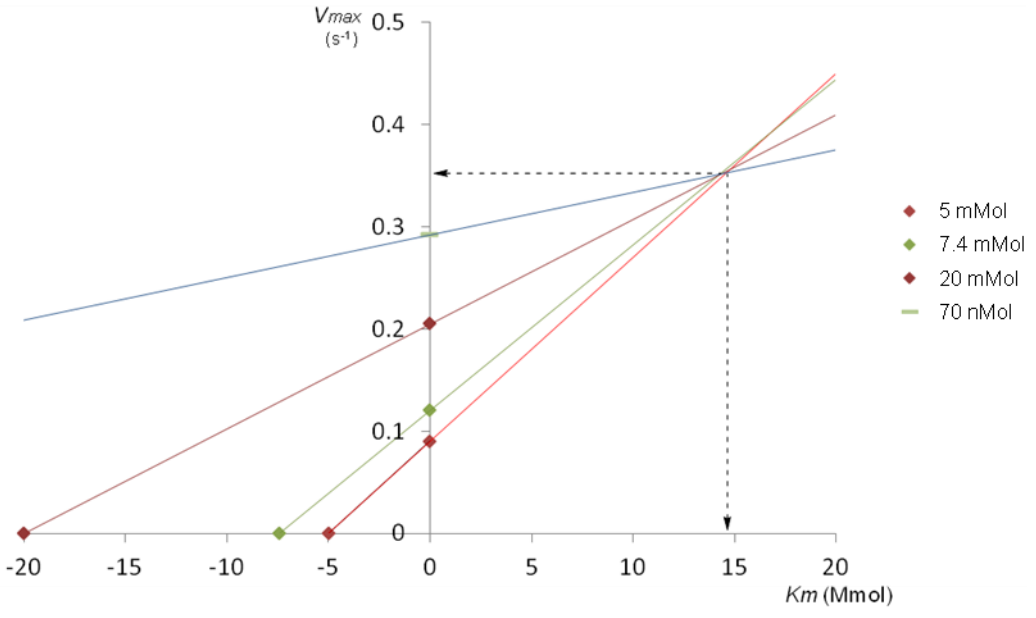
When $V_{max} = 0$ $K_m = -[S]$

Plotting lines through these two points for each sugar concentration gave lines that dissected each other near to one point on the graph, with the x- and y-axis values for this dissection point corresponding to K_m and V_{max}, respectively (Figure 25). The values for K_m and V_{max} may also be determined using other plots derived from the Michaelis-Menten equation, such as the Hanes-Wolfe plot, Lineweaver-Burk and the Eadie-Hofstee plots,[31] however the Direct Linear plot is thought to give a statistically more accurate value for these two kinetic parameters.[30] A Direct Linear plot for determination of K_m and V_{max} for **D-KDX/L-KDA** is presented below (Table 7 and Figure 25). In practice, the Direct Linear plot and analysis were calculated using the Enzpack programme (version 1.4, Elsevier Biosoft), which also reports the values for K_m and V_{max} found using the Eadie-Hofstee, Hanes-Wolfe and Lineweaver-Burk plots for comparison.

Table 7 *TBA assay results for* ***D-KDX/L-KDA***

Entry	1	2	3	4	5	6	7	8
[D-KDX/L-KDA] (mMol)	2.6	5	7.4	10	20	30	50	70
Absorbance	0.056	0.090	0.120	0.129	0.205	0.223	0.235	0.292

Figure 25 *Direct Linear plot of results from* **D-KDX/L-KDA** *(only four results included for clarity)*



The Michaelis-Menten parameters V_{max} and K_m derived from the Direct Linear plot define the catalytic power of an enzyme:

- V_{max} is the maximum velocity when all the enzyme active sites are saturated with substrate and the turnover number (k_{cat}) can be determined from V_{max} when the enzyme mass, concentration and active sites per enzyme are known (Equation 4).

Equation 4 *Rate equation for V_{max}*

$$V_{max} = k_{cat}[E_o]$$

- K_m is made up of three constants (Equation 5) and is inversely proportional to the affinity of the substrate for the enzyme active site (Equations 1 and 6). However, when k_2 is low relative to k_{-1}, K_m is a direct measure of the inverse of the enzyme affinity for the substrate. When K_m is a high value the catalytic efficiency k_{cat} is low (Equation 7).

Equation 5 *Rate constants making-up K_m*

$$K_m = (k_{-1} + k_2) / k_1$$

Equation 6 *Relationship of K_m to enzyme/substrate affinity*

$$K_m \propto k_{-1} / k_1$$

Equation 7 *Catalytic efficiency (k_{cat})*

$$k_{cat} = V_{max} / K_m$$

Michaelis-Menten kinetics were obeyed at 50 °C for both **D-KDX** and **L-KDA**, with kinetic parameters determined using the Direct Linear plot (Table 8). **D-KDX** has a higher K_m value, but this generally lower affinity for the substrate contrasts with a higher turnover number. Consequently, the catalytic efficiencies for both enantiomers are comparable suggesting that KDG aldolase will efficiently catabolise either enantiomer within *S. solfataricus*. When compared to the kinetic parameters previously reported for **D-KDG** and **D-KDGal**, it can be seen that catabolism by KDG aldolase of these pentose sugars is similar in catalytic efficiency to these hexose sugars. This is especially true considering

the fact that the assays for **D-KDG** and **D-KDGal** were originally run at 60 °C and would be expected to give lower values for the turnover number than an assay run at 50 °C [the kinetic parameters for **D-KDX** and **L-KDA** were not recorded at 60 °C as it was thought that the enzyme reaction would proceed at too fast a rate to record an accurate value for the initial rate (*V*)].

Table 8 *Summary of kinetic parameters for KDG aldolase towards* **D-KDX**, **L-KDA**, **D-KDG** and **D-KDGal**

Entry	Substrate	K_m (mM)	k_{cat} (s^{-1})	k_{cat}/K_m ($s^{-1}mM^{-1}$)
1	**D-KDX**[a]	33.3(±3.3)	15.0(±1.1)	0.45(±0.06)
2	**L-KDA**[a]	17.6(±1.1)	9.3(±0.4)	0.53(±0.04)
4	**D-KDG**[b]	25.7(±1.2)	28.2(±1.4)	1.10(±0.08)
5	**D-KDGal**[b]	9.9(±0.4)	6.8(±0.2)	0.70(±0.04)

a. Reactions were carried out at 50 °C in 50 mM sodium phosphate buffer, pH 6.0.
b. Reactions were carried out at 60 °C in 50 mM sodium phosphate buffer, pH 6.0, containing 5 mM fructose-1,6-bisphosphate, 0.2 mM NADH, and an excess of *B. stearothermophilus* L-lactic dehydrogenase (coupled enzyme assay showing the formation of 2-keto-3-deoxy ulosonic acid by the UV absorbance at 340 nm of NADH produced during sugar oxidation).[1]

Therefore, it can be concluded that KDG aldolase is able to not only catalyse catabolism of the diastereomeric C6-sugars **D-KDG** and **D-KDGal**, but also the enantiomeric C5-sugars **D-KDX** and **L-KDA** that are the metabolic intermediates of the two most naturally abundant pentose sugars, D-xylose **8** and L-arabinose **9**. Most metabolic pathways for these sugars are highly conserved, and show excellent stereoselectivity and exclusive specificity profiles for a single sugar isomer.[32] By demonstrating that KDG aldolase exhibits activity towards these enantiomeric sugars, it has been shown that KDG aldolase falls into the smaller category of unselective enzymes that have a broad specificity profile for their natural substrates. When this is placed in context with previous studies on other organisms, it can be concluded that the central metabolic pathway of *S. solfataricus* is inherently "promiscuous" in nature. At this stage it is unclear whether this promiscuity is a survival adaption that the archaeon has evolved to best survive in its extreme environment, or a primitive evolutionary feature that does not occur in higher organisms.[1]

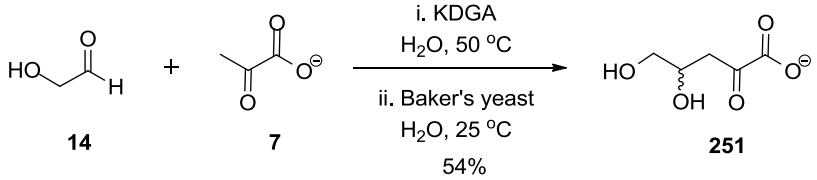
3.8 KDG Aldolase Synthesis of D-KDX/L-KDA

Both **D-KDX** and **L-KDA** were catabolised by KDG aldolase with comparable kinetic parameters, therefore from consideration of the principle of microscopic reversibility it was expected that a forward aldol reaction of pyruvate **7** and glycolaldehyde **14** would produce an approximately racemic aldol product. It was decided to test this hypothesis by conducting a KDG aldolase catalysed reaction between **7** and **14**, and then comparing the optical rotation and CD readings between the enzyme product **251** and the enantiopure products **D-KDX** and **L-KDA**.

Expression and purification of a batch of recombinant KDG aldolase was carried out, in collaboration with Dr. S. F. Royer of the CER at the University of Bath, as described previously using the pET-3a expression vector (Novagen, Nottingham, UK) to insert the KDG aldolase gene into *E.coli* BL21(DE3)(Novagen).[1] After culturing the *E.coli* overnight the KDG aldolase produced was isolated and purified by ion exchange chromatography and gel filtration. This gave KDG aldolase that was homogenous by SDS/PAGE and had a protein concentration of 2.1 mg/ml of water.

This recombinant KDG aldolase was then used to catalyse the aldol reaction of pyruvate **7** and glycolaldehyde **14**, with an excess of pyruvate **7** used to drive the aldol reaction to completion (Scheme 70). When in-process HPLC analysis showed the complete consumption of glycolaldehye **14**, the reaction was quenched by acidification to pH 2 in order to denature the aldolase. After neutralisation of the crude product solution, Bakers yeast expressing pyruvate decarboxylase was added to the reaction mixture to catalyse removal of pyruvate by removal *via* a selective decarboxylation reaction.[33] Purification of the crude reaction product by silica gel chromatography (CH_2Cl_2-MeOH-H_2O, 5:5:1) then furnished the desired product **251** as a yellow oil (363 mg, 54% yield).

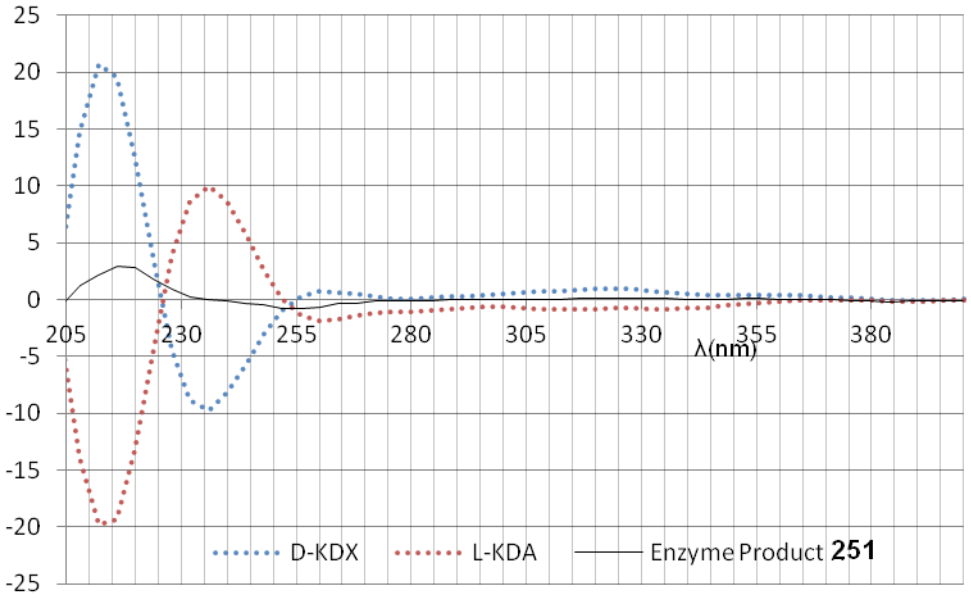
Scheme 70 *KDG aldolase catalysed synthesis of **251***



The isolated product **251** had an optical rotation value of $[\alpha]_D^{26}$ +0.7 (*c* 4.95, H_2O-MeOH, 1:1), which when compared to the optical rotation values of the pure enantiomers (**D-KDX** $[\alpha]_D^{26}$ +13.5; **L-KDA** $[\alpha]_D^{25}$ = -13.3) corresponds to 5% e.e. in favour of **D-KDX**. The ORD results matched the small $[\alpha]_D$ value, with the ORD line of the aldolase-produced product

251 showing only a slight positive curve for **D-KDX**. Thus, it is clear that KDG aldolase exhibits no facial selectivity for the reaction of pyruvate **7** and glycolaldehyde **14**, producing an almost racemic mixture of the two enantiomers **D-KDX** and **L-KDA**, similar to the previously observed unselective aldol reaction of pyruvate **7** with D-glyceraldehyde **6**.[1]

Figure 26 *ORD spectrum of KDG aldolase produced **251**, **D-KDX** and **L-KDA***



The kinetic parameters for the *retro*-aldol cleavage reaction of **251** were also recorded, using the same method as described for the assay of the pure enantiomers **D-KDX** and **L-KDA** (Table 9). As expected, similar *Km*, *kcat* and catalytic efficiency values were recorded for **251**.

Table 9 *Comparison of kinetic parameters of KDG aldolase for **D-KDX**, **L-KDA** and enzymatically produced product **251***

Entry	Substrate	K_m (mM)	k_{cat} (s^{-1})	k_{cat}/K_m ($s^{-1}mM^{-1}$)
1	**D-KDX**[a]	33.3(±3.3)	15.0(±1.1)	0.45(±0.06)
2	**L-KDA**[a]	17.6(±1.1)	9.3(±0.4)	0.53(±0.04)
3	**251**[a]	14.6(±0.8)	11.6(±0.6)	0.79(±0.06)

a. Reactions were carried out at 50 °C in 50 mM sodium phosphate buffer, pH 6.0.

3.9 Conclusion

The first practical method for the synthesis of enantiopure **D-KDX** and **L-KDA** has been developed, using a route which is amenable to the synthesis of other 2-keto-3-deoxy-ulosonic acids. These sugar enantiomers were found to be relatively unstable to acidic, basic and reductive conditions, but can be accessed by treatment of acetonide protected ethyl ester precursors **223** (or **237**) with an aqueous solution carefully acidified with 1 M HCl to pH 1.9, followed by lipase catalysed ester hydrolysis at pH 6.0-7.5. The ^{1}H and ^{13}C NMR spectroscopic data for **D-KDX** and **L-KDA** are reported for the first time, with these sugars having been shown to exist as an essentially 1:1:1 mixture of acyclic, α-furanose and β-furanose isomers in solution. Variable temperature ^{1}H NMR experiments have shown that the isomeric ratio shifts to 51% in favour of the acyclic isomer as the solution temperature is increased from 25 °C to 60 °C; an analogous trend to that previously observed for the C6-2-keto-3-deoxy-ulosonic acid analogues **D-KDG** and **D-KDGal**.

The kinetic parameters for **D-KDX** and **L-KDA** using KDG aldolase have been determined using a modified TBA assay, with good catalytic efficiencies being found for each enantiomer (0.45 and 0.53 $s^{-1}mM^{-1}$). This confirms that *S. solfataricus* uses the same KDG aldolase for the catabolism of not only the diastereotopic C6-sugars **D-KDG** and **D-KDGal** but also for the enantiomeric C5-sugars **D-KDX** and **L-KDA**. Therefore, metabolism of the four most abundant sugars in nature by *S. solfataricus* is largely promiscuous in nature, in stark contrast to the majority of other bacterial and eukaryote metabolic routes, that exhibit highly conserved pathways for individual sugar isomers.

3.10 References

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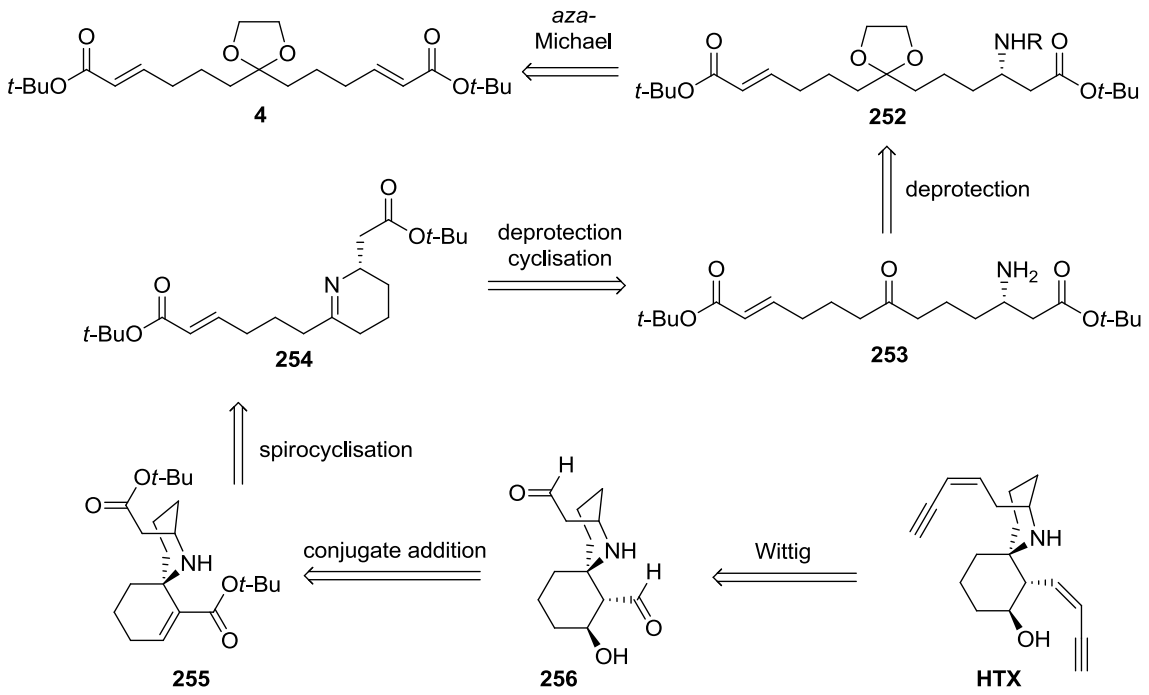
Chapter 4

4.1 Introduction

The second part of this research program was directed towards development of methodology for the total synthesis of Histrinicotoxin **HTX** whose retrosynthesis is shown in Scheme 71, and discussed more fully in the following chapter. As part of this synthesis, an *aza*-Michael reaction was required to convert *bis*-α,β-unsaturated ester **4** into *mono*-β-amino ester **252** in high enantiomeric excess. For this synthesis to proceed it was necessary to develop methodology that would enable stereoselective monoaddition of a nitrogen nucleophile to **4**, with subsequent nitrogen deprotection to afford the primary amine in the presence of a hydrogenolytically sensitive α,β-unsaturated ester group.

Scheme 71 *Proposed aza-Michael addition and amine deprotection for the total synthesis of Histrionicotoxin* ***HTX***



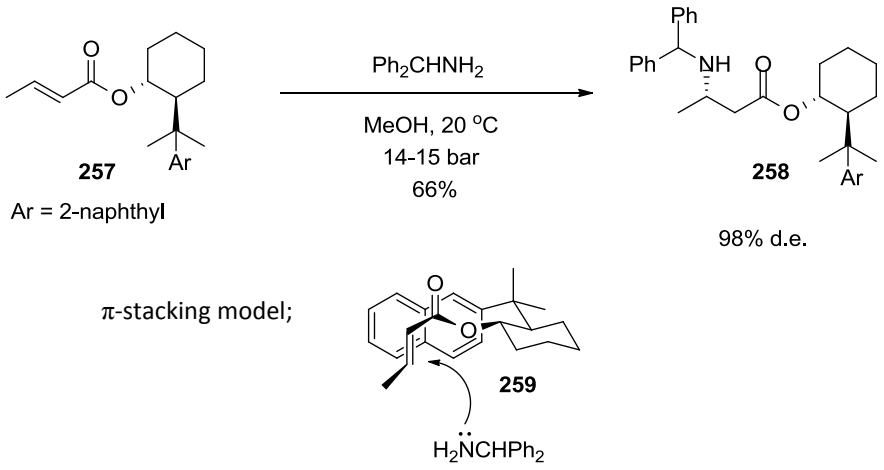
Consequently, recent advances in the asymmetric *aza*-Michael addition of nitrogen based nucleophiles to α,β-unsaturated acid derivatives will now be described highlighting the key synthetic strategies that have been employed, including: addition to a chiral Michael acceptor; addition of a chiral *aza*-nucleophile; chiral metal-complex catalysed addition; chiral Brønsted acid organocatalytic addition; and chiral ligand mediated addition.

4.2 Review of the Asymmetric *aza*-Michael Addition to α,β-Unsaturated Acid Derivatives

4.2.1 *aza*-Michael Addition of Achiral *aza*-Nucleophiles to Chiral Michael Acceptors

There are numerous examples in the literature where researchers have reacted achiral *aza*-nucleophiles with chiral Michael acceptors to afford β-amino acid derivatives.[1-7] Various chiral auxiliaries have been employed to induce stereocontrol into the *aza*-Michael reaction with some excellent diastereoselectivities having been achieved. One of the first examples was reported by the d'Angelo group, where a menthol derived ester was shown to give excellent levels of diastereoselectivity (Scheme 72).[2] They found that it was necessary to conduct the addition reaction under high pressure (>14 bar), which enabled the conjugate addition of diphenylmethanamine to proceed to give **258** in 98% d.e. The authors proposed a π-stacking model **259** to account for the high levels of diastereoselectivity; with the most stable conformation arising from the naphthyl group shielding one face of the crotonate, leading to preferential attack by the amine nucleophile on the less hindered face of the alkene of **257**.

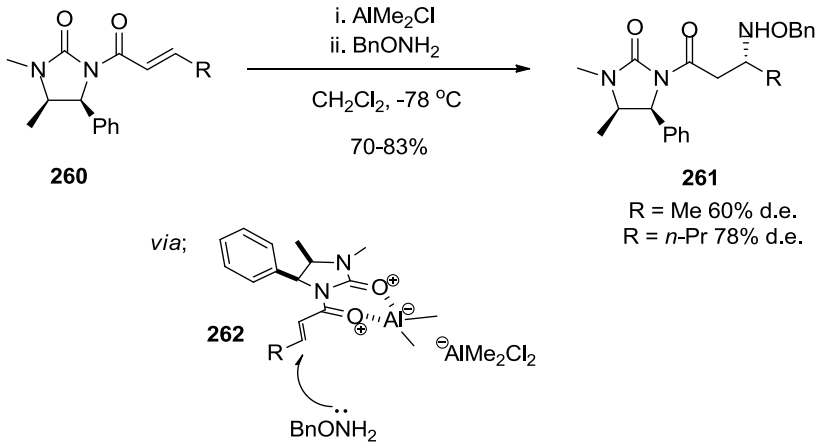
Scheme 72 *Diastereoselective aza-Michael reaction of achiral benzylamine to a chiral crotonate acceptor*



Cardillo and co-workers have demonstrated that the *aza*-Michael addition of *O*-benzylhydroxylamine to chiral imides **260** mediated by a Lewis acid can also furnish β-amino carboxylate derivatives in good diastereoselectivities (Scheme 73).[7] After screening four Lewis acids they found that two equivalents of $AlMe_2Cl$ gave the best results affording **261** in up to 78% d.e. and a yield of 83%. The diastereoselectivity was

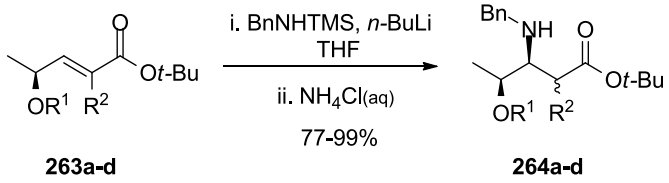
proposed to be the result of Lewis acid chelation to both carbonyls creating a rigid chiral unsaturated system **262**, allowing preferential nucleophilic attack on the less hindered *Re*-face of the alkene group.

Scheme 73 *Diastereoselective aza-Michael reaction of achiral O-benzylhydroxylamine to chiral α,β-unsaturated imide*



Highly stereoselective *aza*-Michael reactions have also been developed that proceed under substrate control. Yamamoto found that stereogenic oxy-substituents in the γ-position of Michael acceptors **263a-d** exert *syn*-diastereocontrol for the conjugate addition of lithium amides (Scheme 74).[6] Increasing the size of the γ-substituent led to higher diastereoselectivities, reaching a *syn*/*anti* ratio of 99:1 for a trityloxy γ-substituent, although the isolated yield was reduced to 77%. They also found that whereas unsubstituted α,β-unsaturated ester **263a** gave low diastereoselectivities, a methyl substituent at the α-position (**263b**) increased the levels of *syn*-selectivity to 100%.

Scheme 74 *Diastereoselective aza-Michael reaction of an achiral aza-anion to a chiral γ-substituted α,β-unsaturated ester*



	R^1	R^2	*syn/anti*
264a	Me	H	54:46
264b	Me	Me	100:0
264c	TBS	H	90:10
264d	*i*-Pr_3C	H	99:1

4.2.2 *aza*-Michael Reactions of Chiral Amide Nucleophiles

As early as 1910, Fischer and Scheibler reported the conjugate addition of ammonia to crotonic acid, and since this initial discovery many amines have been shown to undergo conjugate addition reactions to Michael acceptors.[8-9] The stereoselectivity of this reaction has been widely explored, with mixtures of thermodynamic products often being produced under thermal conditions making high stereocontrol difficult to achieve.[10-11] In contrast the *aza*-Michael addition of metal amides generated by treatment of the corresponding amines with alkyl lithium or Grignard reagents, proceed with good levels of stereocontrol obtained under kinetic control. Early work by Yamamoto and Hawkins revealed that the C_2 symmetric lithium amide **266** underwent highly diastereoselective conjugate addition to *tert*-butyl crotonate (Scheme 75). [11-13] β-amino ester **267** was isolated in 83% yield and an impressive >97% d.e., although deprotection by hydrogenolysis of the nitrogen atom proved difficult, requiring the use of excess palladium hydroxide and hydrogen at elevated temperature. The Furukawa and Davies groups have demonstrated that *aza*-Michael addition of primary amine nucleophiles to α,β-unsaturated ester acceptors gave low yields and poor levels of diastereoselectivity.[10,14] However, Davies went on to demonstrate that the lithium anion of (*R*)-*N*-benzyl-*N*-α-methylbenzylamide **270** reacted with α,β-unsaturated esters to give β-amino esters with very high levels of diastereoselectivity (Table 10). They showed that *tert*-butyl esters gave far superior isolated yields than methyl and benzyl esters (entries 1-3), where competing 1,2-addition pathways reduce the yield of the desired 1,4-addition products. The scope and limitation of this *aza*-Michael addition has been widely explored, with good levels of diastereocontrol observed for *tert*-butyl acceptors containing various aromatic and aliphatic groups at their β-positions, giving β-amino esters with >95% d.e. in all cases (entries 3-6). Use of lithium amides of secondary amines containing two chiral centres, such as (*R*)-*bis*((*R*)-1-phenyl-ethyl)-amine, gave high levels of diastereocontrol (>97% d.e.), but attenuated reaction rates and yields.

Scheme 75 *Hawkins aza-Michael addition methodology of a chiral binaphthyl aza-anion to a crotonate tert-butyl ester Michael acceptor*

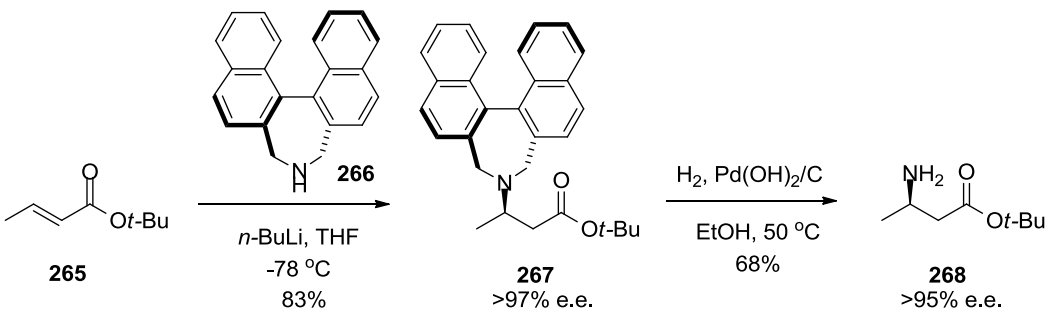
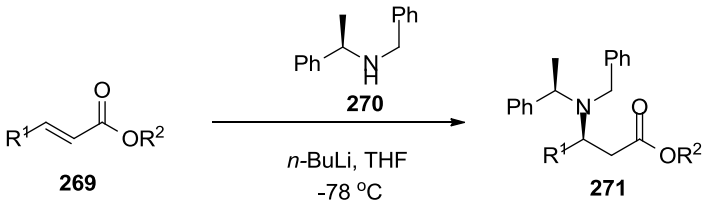


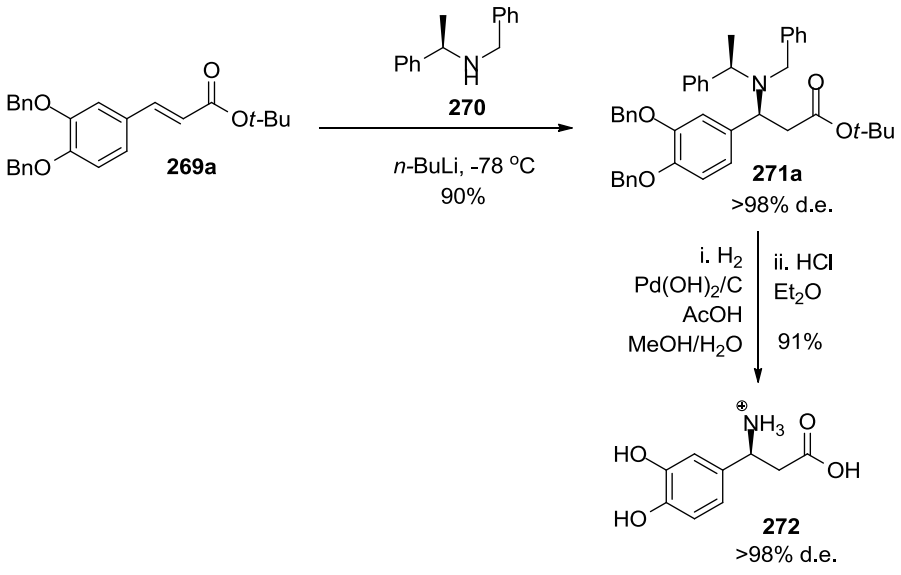
Table 10 *Diastereoselective aza-Michael reaction of a chiral lithium amide to an α,β-unsaturated ester*



Entry	R^1	R^2	Yield	d.e. (%)
1	Ph	Me	68	94
2	Ph	Bn	39	91
3	Ph	*t*-Bu	96	>95
4	2-$MeOC_6H_4$	*t*-Bu	95	>95
5	Et	*t*-Bu	92	>95
6	*n*-Pent	*t*-Bu	81	>95

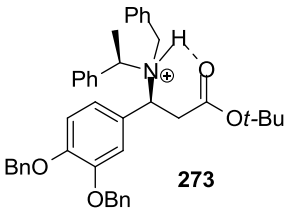
This methodology has been used in the synthesis of a large number of pharmaceutically active compounds and natural products.[14-17] For example, (*R*)-3,4-dihydroxy-β-phenylalanine **272**, a naturally occurring β-amino acid was synthesised *via aza*-Michael addition of lithium amide **270** to α,β-unsaturated ester **269a**, followed by global benzyl deprotection by hydrogenolysis and acid promoted hydrolysis to give **272** in >98% d.e. and in an overall 82% yield (Scheme 76).[14]

Scheme 76 *Synthesis of (R)-3,4-dihydroxy-β-phenylalanine **272***



It is interesting to note that, unlike the other two benzylic bonds, the C(3)-N benzylic bond is not cleaved under the hydrogenolysis conditions used, which has been found to be the general rule for hydrogenolysis of this type of β-amino acid. It has been postulated that this selectivity arises from formation of a protonated ammonium intermediate **273** that holds the C(3)-β-aryl group in a conformation that disfavours hydrogenolysis and cleavage of the C(3)-N bond (Figure 27).

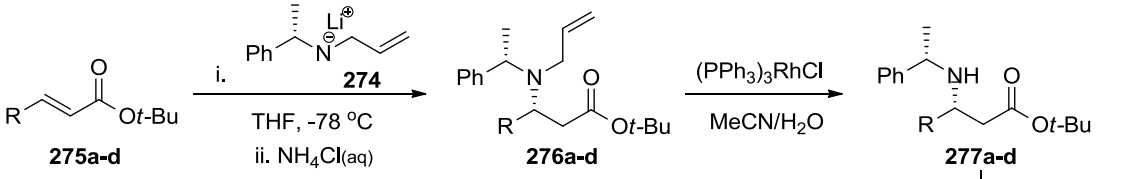
Figure 27 *Postulated protonated ammonium intermediate*



273

Although the *aza*-Michael addition of (*R*)-*N*-benzyl-*N*-α-methylbenzylamide **266** proceeds with excellent stereoselectivity, a drawback to this metholodogy is the necessity of using hydrogenolytic conditions to remove both benzyl protecting groups that is incompatible with other hydrogenolytically sensitive functional groups such as alkenes. To overcome this drawback Davies and Fenwick demonstrated that the lithium ion of (*S*)-(α-methylbenzyl)allylamine **274** also undergoes highly diastereoselective *aza*-Michael additions to α,β-unsaturated *tert*-butyl esters (Scheme 77).[18] The β-amino ester products **276a-d** could then be efficiently deallylated with $(PPh_3)_3RhCl$ (Wilkinson's catalyst) and converted, after transesterification to methyl esters, to the corresponding azetidin-2-ones **278a-d** by treatment with methyl magnesium bromide.

Scheme 77 *aza-Michael addition of lithium (R)-N-benzyl-N-α-methylbenzylamide **274** to α,β-unsaturated tert-butyl esters with alternative rhodium catalysed deprotection protocol*

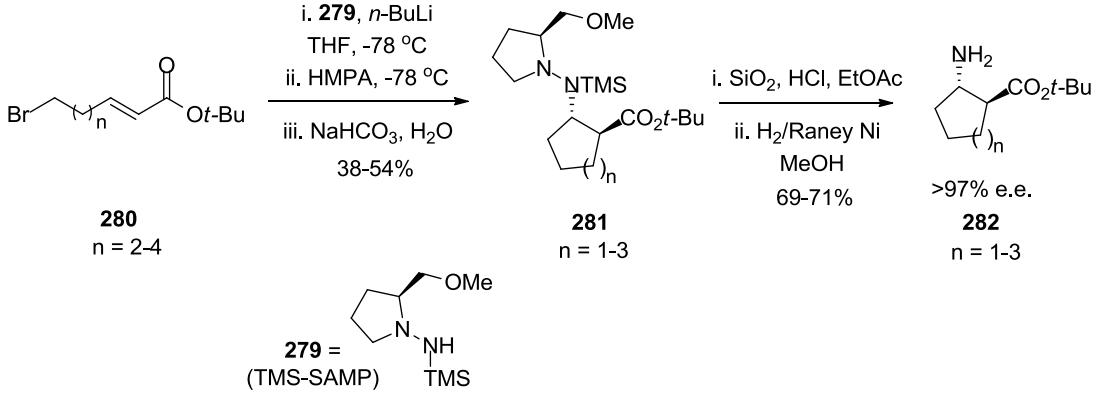


	R	% Yield[a]	% d.e.
278a	Me	68	100
278b	Et	58	100
278c	Ph	72	100
278d	2-furyl	64	100

a. yield for three steps

Of the other homochiral "ammonia equivalents" investigated, conjugate addition of the lithium anion of (*S*)-(-)-2-methoxymethyl-1-trimethylsilylaminopyrrolidine (TMS-SAMP) **279** has given particularly impressive results. The Enders group showed that **279** underwent highly diastereoselective addition to a range of β-alkyl substituted α,β-unsaturated *tert*-butyl esters to give *aza*-Michael products with 93-98% d.e.[19] They subsequently demonstrated this methodology for the synthesis of cyclic β-amino esters **281**, where the intermediate ester enolates reacted intramolecularly with a ω-carbon-halide bond resulting in ring closure (Scheme 78).[20] Desilylation using silica in ethyl acetate with trace amounts of HCl, followed by amine deprotection using H_2/Raney Ni furnished the desired cyclic β-amino esters **282** in high enantiomeric excess.

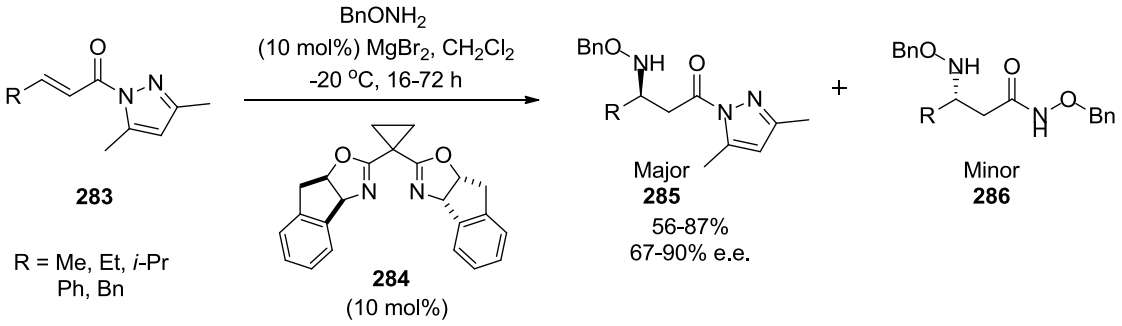
Scheme 78 *Diastereoselective aza-Michael addition of the lithium ion of TMS-SAMP **279** to β-alkyl substituted α,β-unsaturated tert-butyl esters*



4.2.3 Metal Catalysed Asymmetric *aza*-Michael Addition Reactions

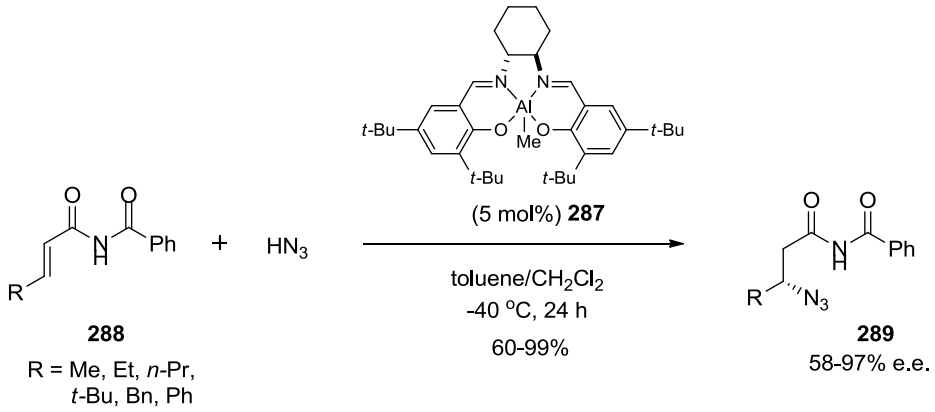
An early example of a metal-catalysed asymmetric *aza*-Michael reaction was reported by the Sibi group in 1998.[21] They reacted *O*-benzylhydroxylamine with α,β-unsaturated pyrazole amides **283** using catalytic quantities of a chiral Lewis Acid prepared from $MgBr_2.OEt_2$ and bisoxazoline **284** (Scheme 79). Good to excellent enantioselectivities were achieved for amidic acceptors containing aliphatic β-substituents, but aromatic groups in the β-position gave low yields and poor enantioselectivities. When the catalyst loading was increased to 30% and then to 100%, a decrease in yield, and a contrasting increase in enantioselectivity was observed. After noticing that there was a concomitant increase in by-product **286** over time, that is formed by amidolysis of product **285**, Sibi suggested that kinetic resolution was occurring *via* preferential amidolysis of the opposite enantiomer of **285**.

Scheme 79 *Aza-Michael reaction of O-benzylhydroxylamine to α,β-unsaturated amides catalysed by a chiral magnesium catalyst*



As part of their work studying reactions catalysed by metal complexes of chiral salen ligands, the Jacobsen group screened the *aza*-Michael addition reactions of hydrazoic acid in the presence of (salen)Al(III) complexes such as **287**.[22] They found that hydrazoic acid underwent highly enantioselective *aza*-Michael reactions with α,β-unsaturated imides **288**, affording β-*aza*-imides **289** in 95-97% e.e., except for a phenyl substituted derivative that gave an *aza*-Michael product of only 58% e.e. (Scheme 80).

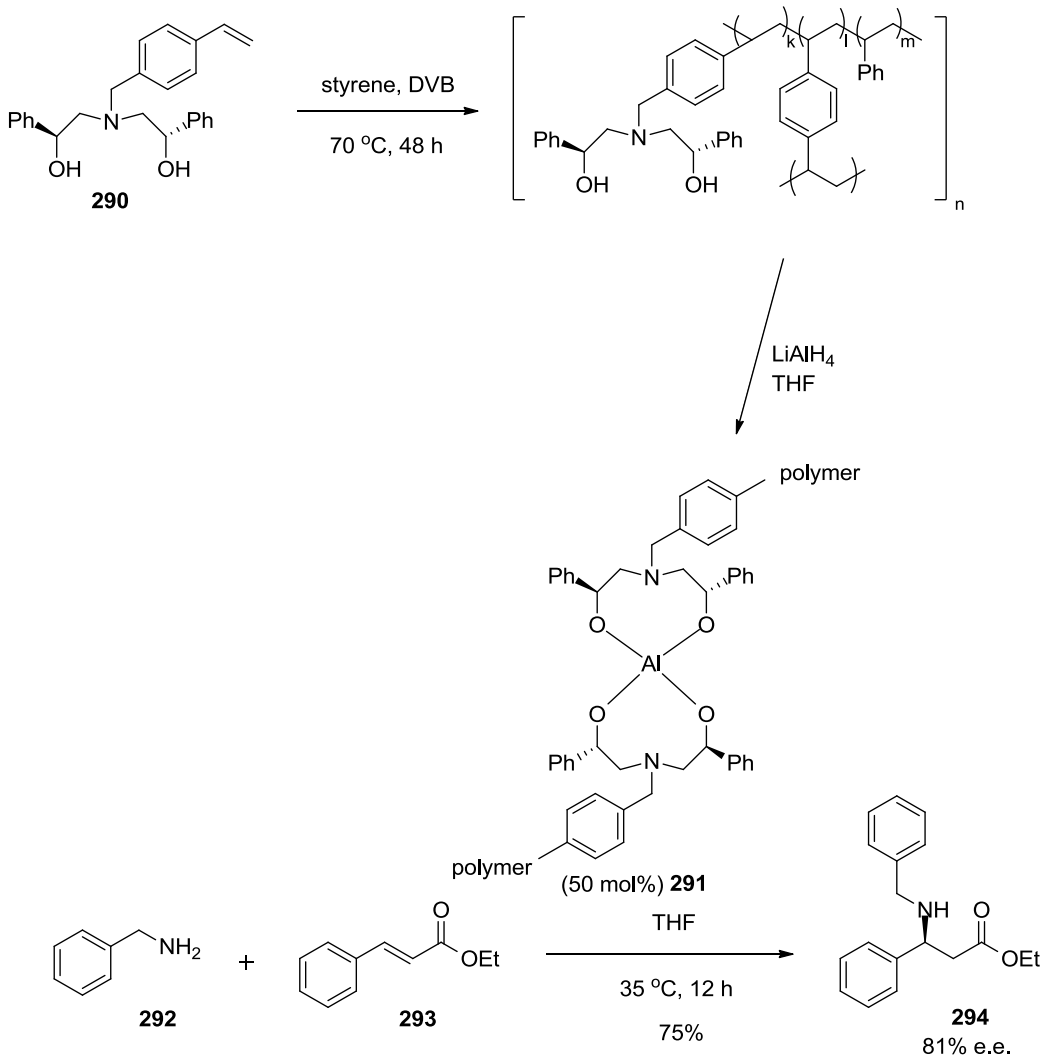
Scheme 80 *aza-Michael reaction of hydrazoic acid with imides catalysed by (salen)Al(III)*



Sundararajan and Prabagaran have endeavoured to develop a polymer supported chiral catalyst for enantioselective *aza*-Michael reactions, with the aim of simplifying purification of reaction mixtures (Scheme 81).[23] The chiral polymer was formed by free radical copolymerisation of divinylbenzene (DVB), styrene and the chiral amino-diol **290**. This polymer was then complexed with aluminium by simply stirring an excess of polymer with $LiAlH_4$ in THF under an inert atmosphere. Good levels of enantioselectivity were then

achieved for the reaction of benzylamine **292** and ethyl cinnamate **293**, but when the catalyst was recycled between each run, reduced enantioselectivities were achieved and the polymer was found to deteriorate to a fine dust due to attrition by mechanical stirring. Despite these problems, this work demonstrates that polymer supported chiral catalysts have the potential to act as efficient catalysts, for stereoselective *aza*-Michael additions.

Scheme 81 *Catalysis of the aza-Michael reaction by chiral polymer aluminium catalyst*

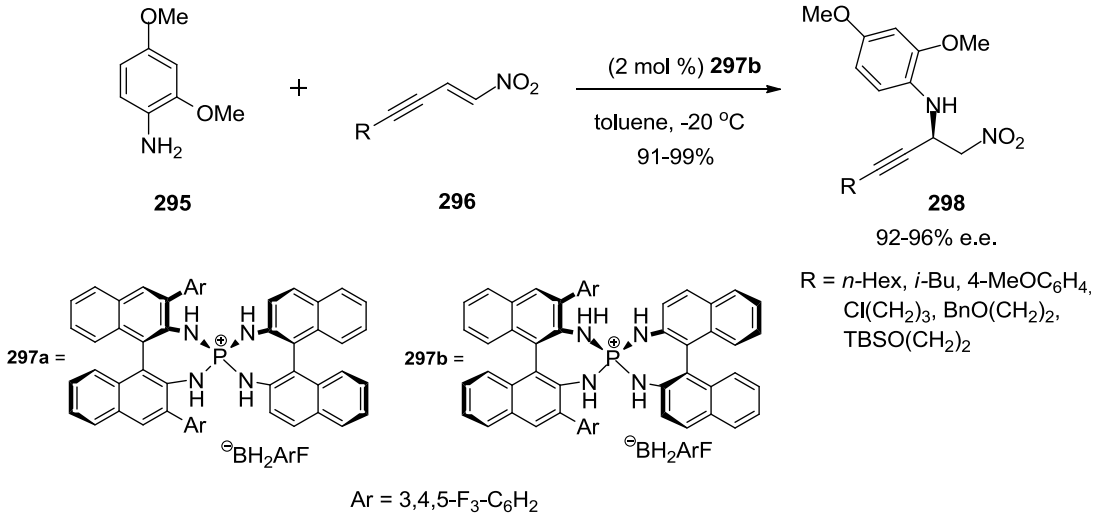


4.2.4 Brønsted Acid and Organocatalytic Approaches to the Asymmetric *aza*-Michael Reaction

Recently a few examples of Brønsted acid catalysed *aza*-Michael reactions to Michael acceptors have been reported using phosphorous and thiourea based catalysts. The Ooi

group reported the use of chiral arylaminophosphonium barfate catalysts for the *aza*-Michael reaction of 2,4-dimethoxyaniline **295** to nitrostyrenes with high enantio-selectivities.[24] More recently they have extended this work to addition reactions of 2,4-dimethoxyaniline to nitro-enynes **296** (Scheme 82).[25] Initial trials using catalyst **297a** at room temperature gave good yields with low enantioselectivites, but this was greatly improved by conducting the reaction at lower temperatures, and using the binaphthyl catalyst **297b**. Under these conditions a range of β-amino homopropargylic nitro compounds **298**, with variance of the electronic and steric properties of the R-group, were synthesised in high yield and excellent regio- and enantioselectivities.

Scheme 82 *Chiral arylaminophosphonium barfate **297a** catalysed aza-Michael reaction of **295** to enyne-nitro Michael acceptors **296***



The use of weakly acidic chiral thioureas has been explored by Sibi and co-workers affording good results for the *aza*-Michael reaction of *O*-substituted hydroxylamines **300** to pyrazole crotonates **283** (Scheme 83).[26] The optimised conditions yielded the desired product in mostly good yields with good to excellent levels of enantioselectivity. The reaction proceeds using 0.3 molar equivalents of catalyst **301**, but the authors preferred to use one equivalent to increase the rate of reaction. By varying the substrate and catalyst structure and observing how the yields and enantioselectivities of product were affected, it was concluded that the chiral thiourea **301** was acting as a bifunctional catalyst to facilitate addition (Figure 28).

Scheme 83 *Thiourea catalysed aza-Michael reaction of O-substituted hydroxylamines **300** to α,β-unsaturated amide Michael acceptors **283***

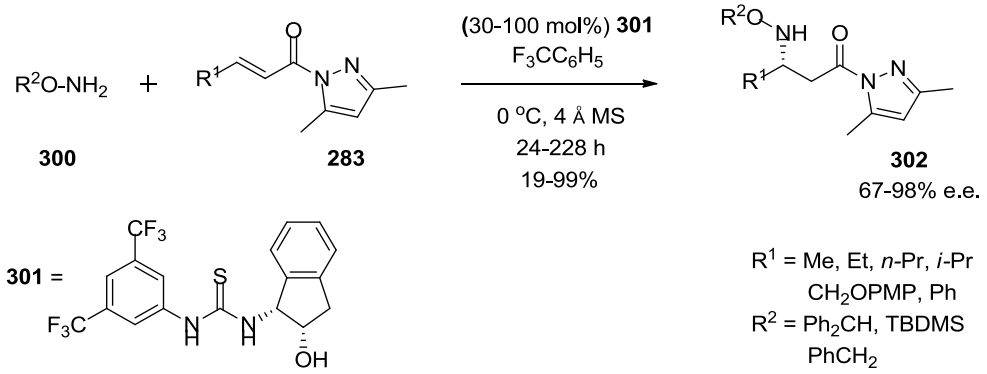
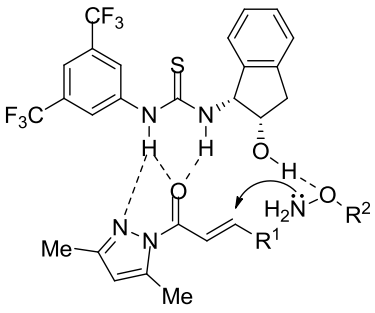


Figure 28 *Proposed transition state model for chiral thiourea catalysed aza-Michael reactions*

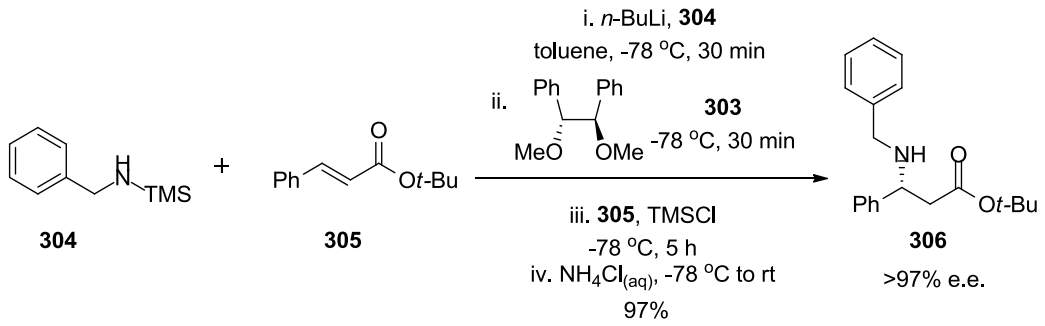


4.2.5 Chiral Ligand Mediated Asymmetric *aza*-Michael Reactions

Tomioka and co-workers have demonstrated that chiral diether ligand **303** can induce high levels of stereocontrol into the *aza*-Michael reaction of lithium amides to α,β-unsaturated ester derivatives (Scheme 84).[27] The silyl-amine **304** was first deprotonated using *n*-butyl lithium at -78 °C before addition of chiral diether ligand **303**, and stirring at -78 °C for 30 minutes to allow for complexation to occur. A toluene solution of *tert*-butyl crotonate **305** and TMSCl was then added dropwise and the reaction was stirred at low temperature for five hours. The *N*-protected-β-amino ester **306** was obtained in an impressive 97% e.e.

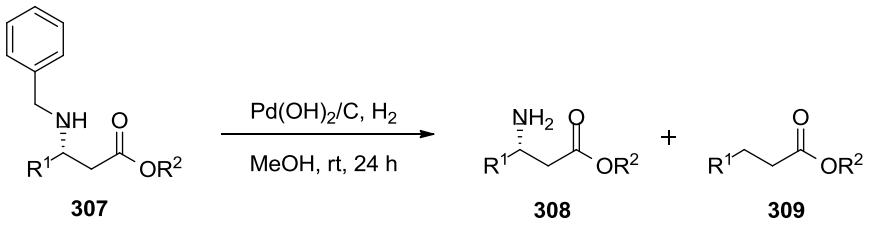
and 97% yield. This methodology was shown to work equally well using methyl, *iso*-propyl, alkenyl and naphthyl β-substituents in place of the phenyl group.

Scheme 84 *Chiral diether ligand **303** mediated aza-Michael reaction*



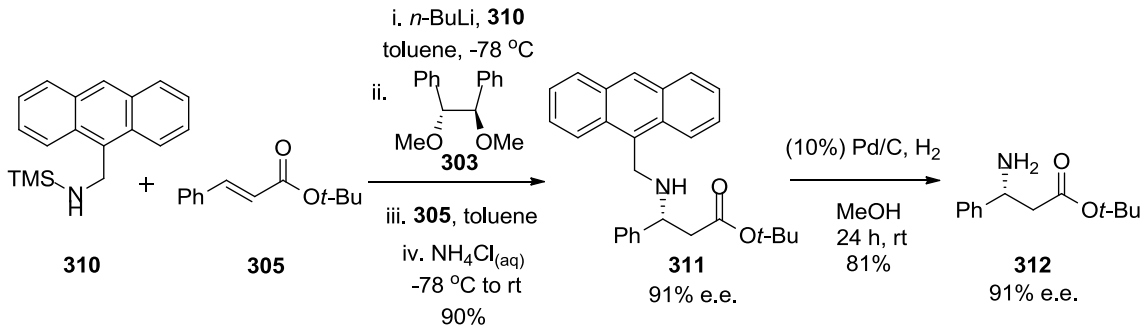
The Tomioka group has gone on to show that other silyl amines could be successfully employed as *aza*-nucleophiles in this type of asymmetric *aza*-Michael addition reaction (Scheme 86i-iii).[28-30] They tested these alternatives primarily to find an ammonia equivalent that could be deprotected using conditions other than hydrogenolysis, since whilst being incompatible with the presence of some functional groups, hydrogenolysis was shown to sometimes lead to the undesired cleavage of the C(3)-N to give 3-phenylpropanoate **309** type products (Scheme 85). Anthraceny-9-ylmethyl amine **410** gave improved yields for deprotection under milder hydrogenolytic conditions, but it still required 24 hours using 10% Pd/C (cf. 41 hours for deprotection of benzyl amine **306** under these conditions) (Scheme 86i).[30] Allylic amine **314** could be deprotected by rhodium catalysed isomerisation (Scheme 86ii),[28] whilst the mesitylmethylamine **317** could be deprotected by a protracted three step chlorination/regioselective dehydrochlorination/transoximation strategy (Scheme 86iii).[29] Unsuccessful attempts were made to use an ammonia equivalent that could be deprotected under oxidative conditions, with *N*-silyl protected *para*-methoxybenzylamine **325** being reported to be unsuccessful in the *aza*-Michael reaction in their hands, *vide infra*.[30]

Scheme 85 *3-Phenylpropanoate **309** by-product formed by hydrogenolytic cleavage of C(3)-N bond*

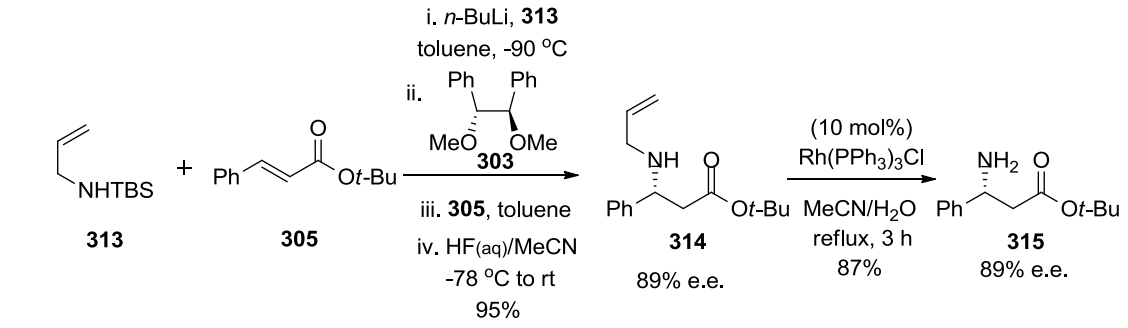


Scheme 86 *Alternative silyl amides employed as aza-nucleophiles for the chiral ligand mediated aza-Michael reaction*

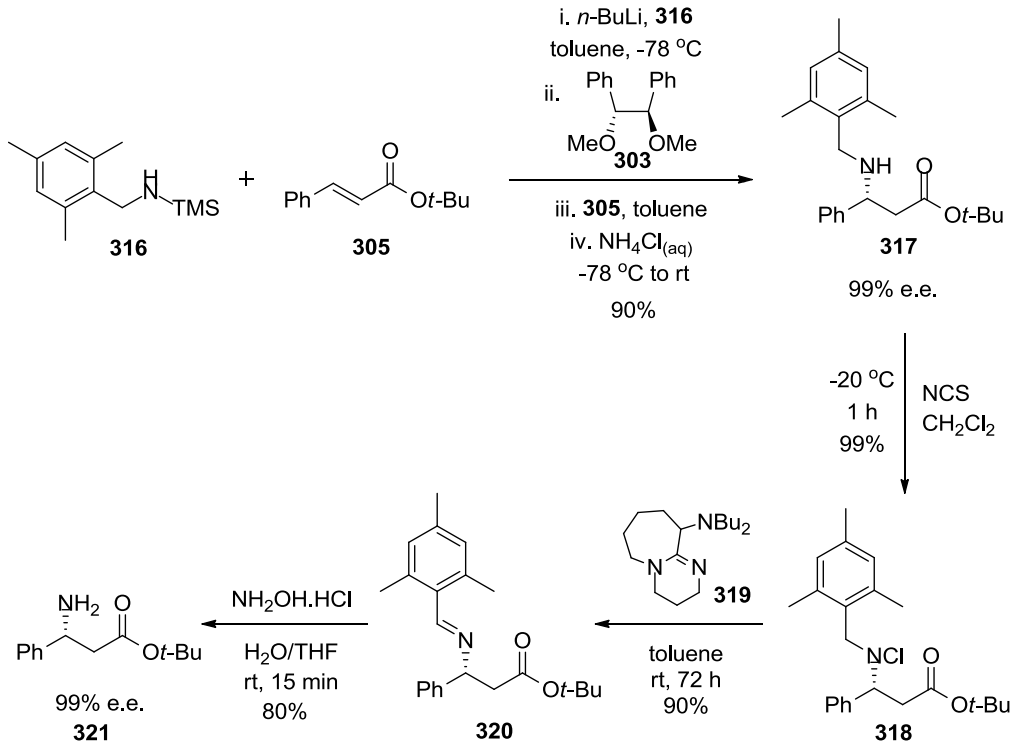
(i)



(ii)

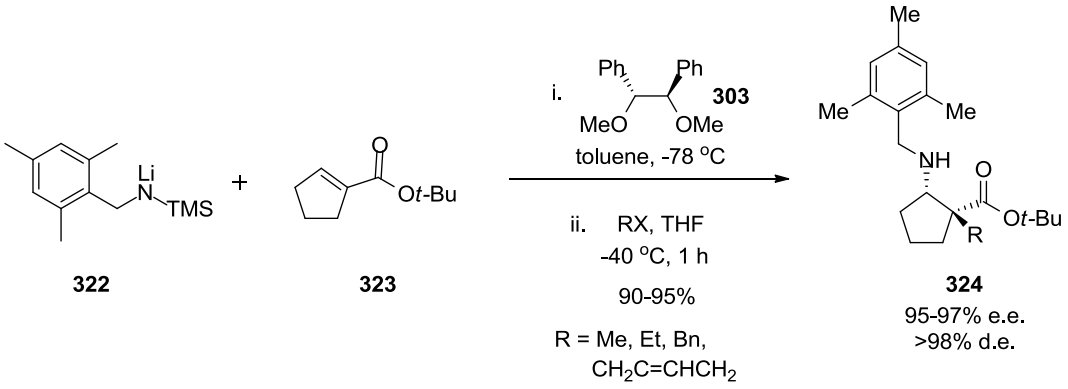


(iii)



Tomioka went on to develop tandem enolate alkylation reactions using this methodology (Scheme 87),[31] with chiral diether ligand **303** controlling the enantioselectivity of addition of **322** to Michael acceptor **323**, followed by *in situ* alkylation at the α-position with a range of electrophiles, to give quaternary carbon β-amino esters **324** in high enantiopurity.

Scheme 87 *Chiral diether ligand controlled aza-Michael addition tandam enolate alkylation reaction*



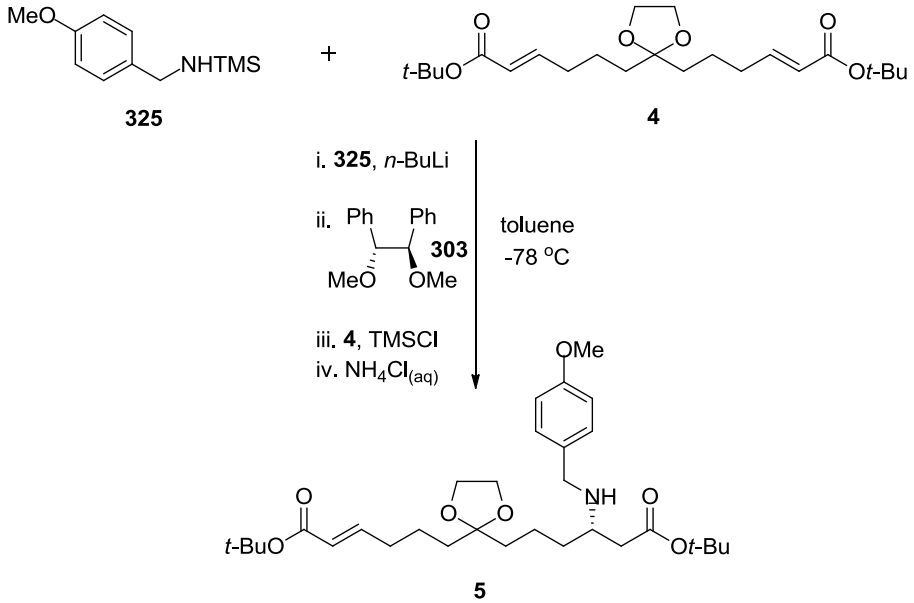
322 **323** **324**

95-97% e.e.
>98% d.e.

4.3 Investigation of the Asymmetric *Aza*-Michael Reaction of *N*-(4-methoxybenzyl)-1,1,1-trimethylsilanamide

It was decided to explore *aza*-Michael reaction mediated by Tomioka's chiral diether ligand **303** as a means of stereoselectively introducing nitrogen functionality into C(3) of *bis*-α,β-unsaturated ester **4** for the total synthesis of Histrionicotoxin **HTX** (Scheme 88).

Scheme 88 *Proposed aza-Michael addition mediated by chiral diether ligand **303***

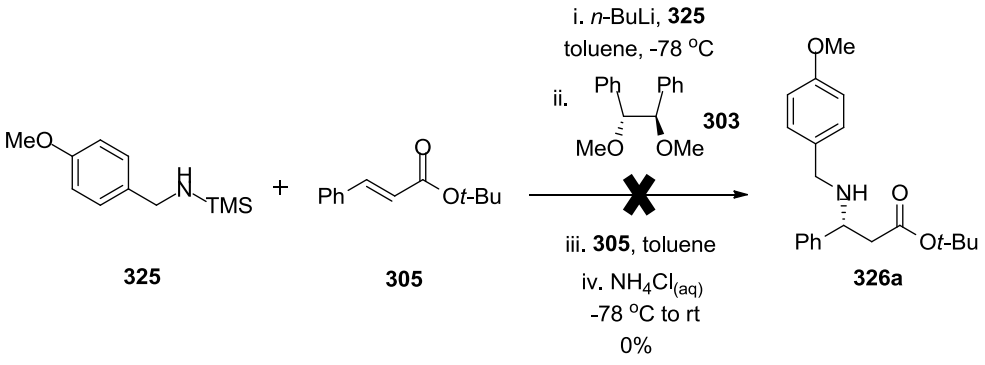


325 **4**

5

Because Tomioka's chiral ligand methodology had been shown to add *N*-trimethylsilylbenzylamine **304** to simple α,β-unsaturated esters in good yield and excellent enantioselectivity, this was chosen as the starting point for synthetic investigations.[27] However, the main drawback to using Tomioka's methodology was that deprotection of the *N*-benzyl protecting group required hydrogenolysis, which would also reduce the remaining conjugated alkene bond of **5**. As described, Tomioka and co-workers screened the *aza*-Michael reactions of various other ammonia equivalents, whose adducts would be deprotected by methods other than hydrogenolysis.[28-30] As part of these studies, the lithium ion of *N*-(4-methoxybenzyl)-1,1,1-trimethylsilanamine **325** was explored as a nucleophile, whose *aza*-Michael addition products could potentially be deprotected using oxidants such as DDQ and CAN. However, it was reported that attempts to employ this nucleophile resulted in none of the desired *aza*-Michael product using *tert*-butyl cinnamate **305** as the Michael acceptor (Scheme 89). The authors speculated that the lack of addition product formed in these reactions was due to "too much bulkiness or poor nucleophilicity" of the nitrogen nucleophile **325**. However, the "bulkiness" of **325** was certainly no greater than mesitylmethylamine **316** that they reported as a good nitrogen nucleophile for this type of *aza*-Michael reaction,[29] whilst the presence of the *para*-methoxy substituent would not have been expected to decrease the nucleophilicity of the lithium amide significantly.

Scheme 89 *Tomioka attempted aza-Michael addition of N-(4-methoxybenzyl)-1,1,1-trimethylsilanamine **325** to tert-butyl cinnamate **305***



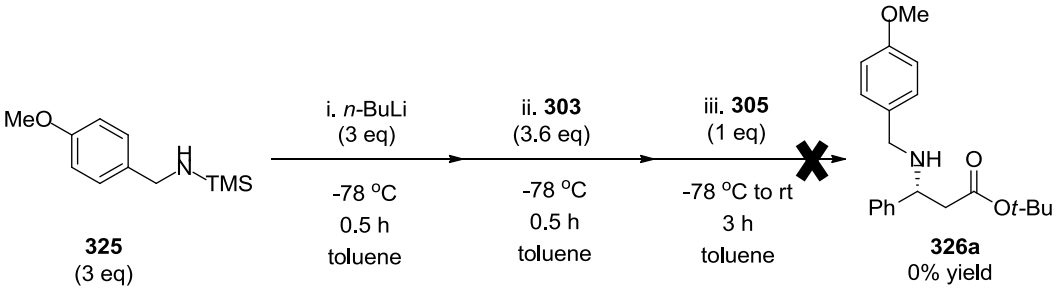
325 **305** **326a**

Significantly, in the course of investigation into the *aza*-Michael addition of chiral 2° amines to α,β-unsaturated esters (Section 4.2.1), Davies and co-workers reported the *aza*-Michael addition of the lithium anion of (*S*)-*N*-benzyl-1-(4-methoxyphenyl)ethanamine to *tert*-butyl cinnamate **305**, giving excellent levels of stereoselectivity and good yields of

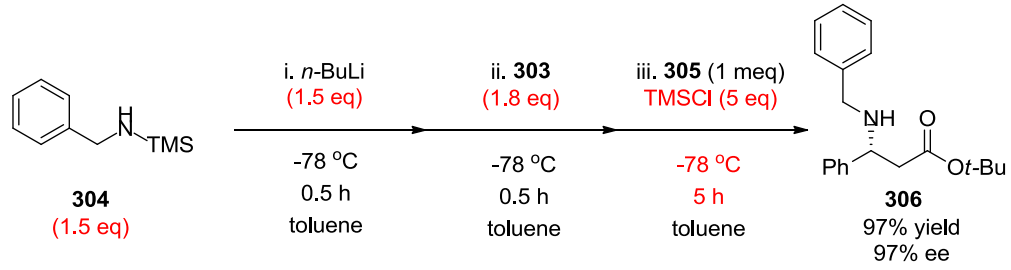
β-amino acid product **329** after subsequent treatment with CAN and HCl(aq). (Scheme 91).[32] Close inspection of the unsuccessful *aza*-Michael protocol employed by Tomioka for the *aza*-Michael addition of amine **325** revealed that the reaction conditions employed were not the optimised protocol reported previously for the successful *aza*-Michael reaction of amine **304**, with differences in the number of equivalents of amine base employed, length of reaction time, reaction temperature, and most importantly the absence/presence of TMSCl (Scheme 90i and ii).

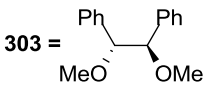
Scheme 90 *Comparison of the reaction protocol employed by Tomioka for: (i) the unsuccessful aza-Michael reaction of aza-nucleophile* ***325*** *with tert-butyl cinnamate* ***305****; (ii) the successful aza-Michael reaction of aza-nucleophile* ***304*** *with tert-butyl cinnamate* ***305*** *(differences indicated in red)*

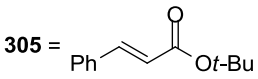
(i)



(ii)

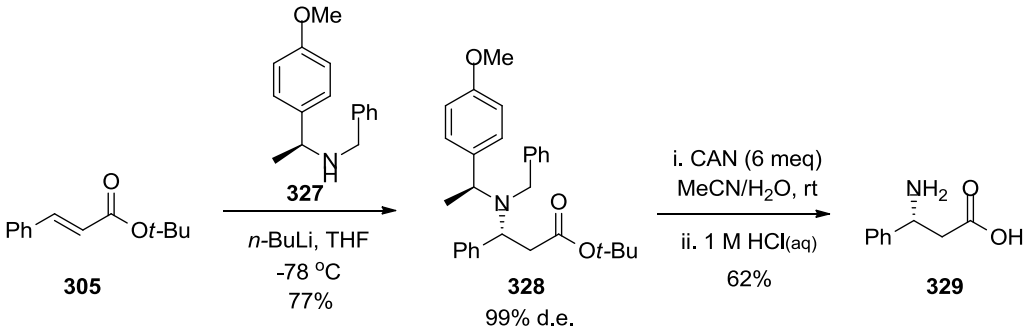






Therefore, it was proposed that carrying out the *aza*-Michael reaction of amine **325** with *bis*-α,β-unsaturated ester **4** under more optimal conditions might afford the desired *mono*-addition product **5** in high yield and enantioselectivity.

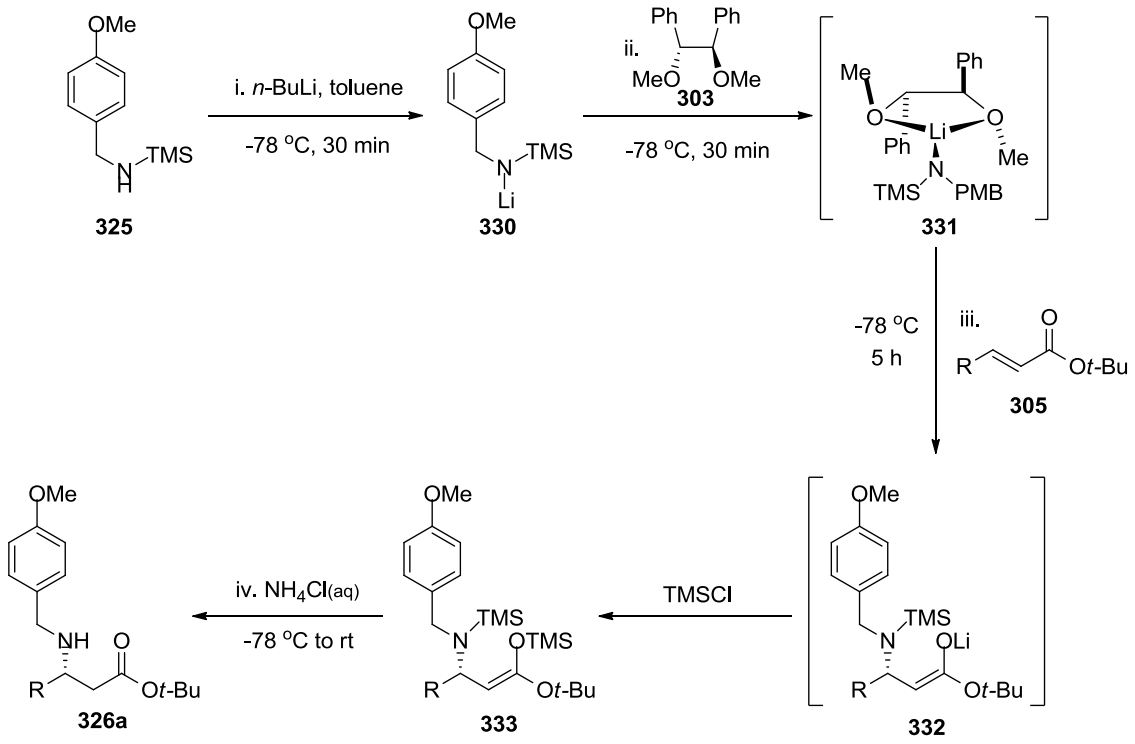
Scheme 91 *Davies successful aza-Michael addition of chiral 2° amine* ***327*** *to tert-butyl cinnamate* ***305***



4.4 Investigation of the Asymmetric *aza*-Michael Reaction of *N*-(4-Methoxybenzyl)-1,1,1-trimethylsilanamide to a *bis*-α,β-Unsaturated Ester

Initially, the *aza*-Michael reaction of the lithium anion of amine **325** with *bis*-α,β-unsaturated ester **4** mediated by chiral diether ligand **303** was investigated using the same conditions and molar equivalents of TMSCl additive that Tomiaka had used for the successful *aza*-Michael reaction of **304** shown in Scheme 90ii. The detailed mechanism for the chiral diether ligand **303** mediated reactions of lithium amides has yet to be fully established, but it is thought to proceed *via* formation of a monomeric lithium amide complex **331** *in situ* (Scheme 92).[33] An α,β-unsaturated ester **305** then coordinates to the lithium atom of complex **331** minimising steric repulsion with the two methyl groups of the ligand, resulting in enantioselective intramolecular nucleophilic attack of **325** to afford a lithium enolate **332**, which reacts in turn with the excess TMSCl to give (*Z*)-silylketene acetal **333**. The use of TMSCl to trap the enolate prevents the enolate interfering with formation of complex **331**, which has previously been blamed for lowered enantioselectivites and yields of *aza*-Michael products.[27] Furthermore, it may also function to prevent the *retro-aza*-Michael reaction of **332** that would result in starting materials being regenerated. Treatment with saturated aqueous ammonium chloride during work-up then hydrolyses the *N*-Si and *O*-Si bonds to afford the desired β-amino ester products.

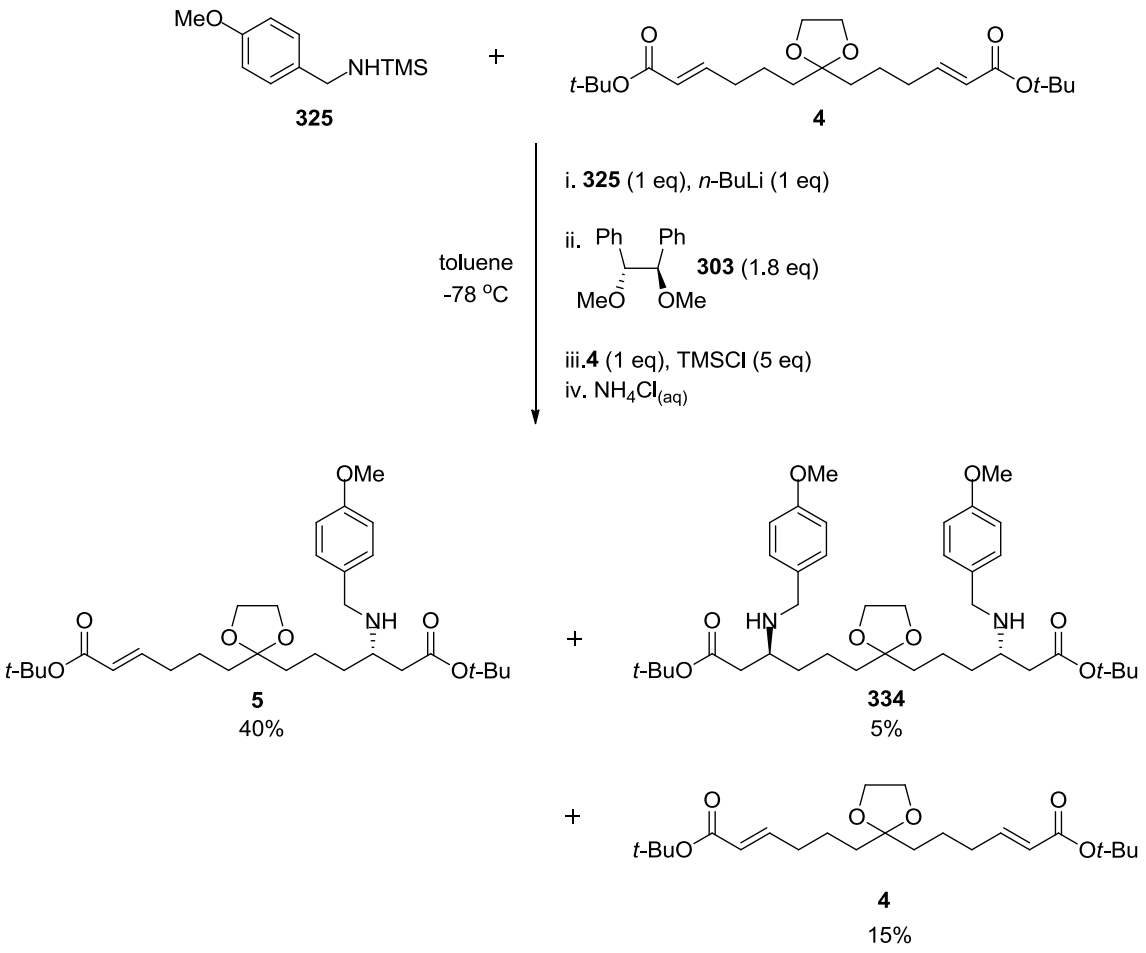
Scheme 92 *Mechanism of the chiral diether ligand **303** mediated aza-Michael reaction*



Therefore, a dry solution *n*-butyllithium (2.6 M in hexanes) in toluene was cooled to -78 °C and a dry solution of amine **325** in toluene was added dropwise and the reaction mixture stirred for 30 minutes. Then chiral ligand **303** dissolved in dry toluene was added dropwise and the reaction mixture stirred for a further 30 minutes. The reaction mixture was then transferred *via* an insulated cannula to a solution of *bis*-α,β-unsaturated ester **4** and TMSCl in dry toluene and stirred at reduced temperature for five hours. After quenching the reaction mixture with a saturated aqueous solution of ammonium chloride, a standard work-up procedure furnished a yellow oil that was purified by silica gel chromatography to afford the mono-addition product in an isolated yield of 40%, with the major impurities isolated in separate fractions and identified as unreacted starting material **4** (15%) and *bis*-addition product **334** (5%) (Scheme 93).

Since the chiral ligand **303** mediated *aza*-Michael reaction of silylamines generally proceed with excellent enantioselectivities of >97% e.e., it was hoped that the *aza*-Michael reaction of **325** would be equally enantioselective. To determine the enantiomeric excess of the reaction, the *aza*-Michael reaction of amine **325** with **4** was repeated in the absence of chiral ligand **303**, which after purification furnished pure racemic *mono*-addition product *rac*-**5**.

Scheme 93 *Aza-Michael addition of N-(4-methoxybenzyl)-1,1,1-trimethylsilanamine* ***325***



In order to distinguish between the two enantiomers of *rac*-**5**, a number of chiral HPLC columns were screened for their ability to resolve its enantiomers: Hichrom C18; Lux Cellulose 1; Regis Whelk-O 1; and Kromasil AmyCoat. Excellent results were obtained with the Kromasil 5 µm AmyCoat stationary phase eluting with an isocratic gradient of isohexane/IPA (95/5) + 0.15% DEA at 1ml/min, which gave baseline separation of the two enantiomers of *rac*-**5** (Figure 29). A sample of **5** prepared in the presence of chiral ligand **303** was then run using this method (Figure 30), which revealed that **5** had been formed with an enantiopurity >97% e.e., indicating that the *aza*-Michael reaction had proceeded with excellent levels of enantiocontrol. The absolute configuration of **5**, inferred from Tomioka's previous reports of the *aza*-Michael reaction of lithium amides mediated by chiral ligand **303**, was assigned to be the (*S*)-enantiomer.

Figure 29 *HPLC trace of rac-**5** run on a Kromasil 5-AmyCoat chiral column*

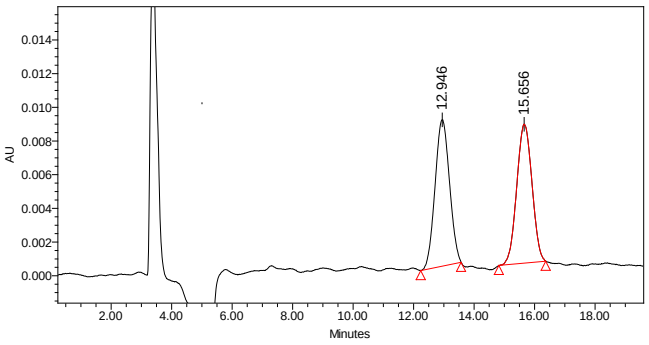
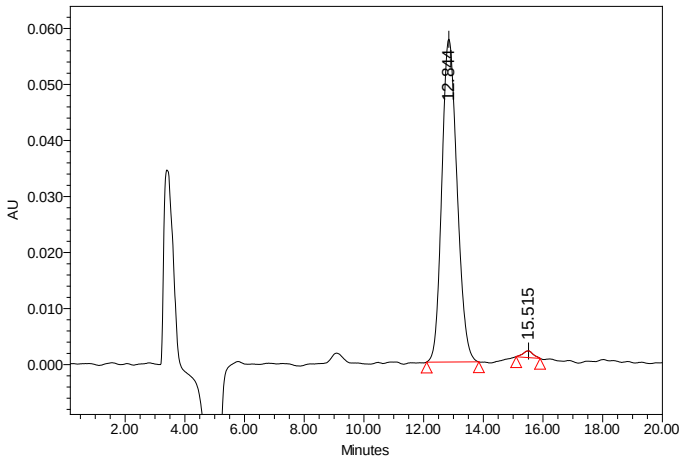


Figure 30 *HPLC trace of enantiopure **5** (>97% e.e.) run on a Kromasil 5-AmyCoat chiral column*



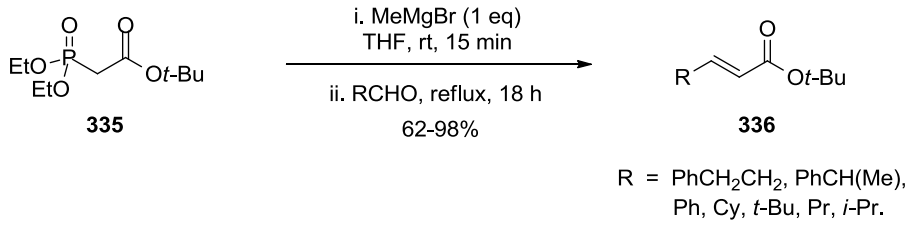
Having succeeded in the synthesis of **5** *via* a highly stereoselective *aza*-Michael reaction, this *mono*-addition reaction was subsequently utilised to prepare gram quantities of **5** for the total synthesis of Histrionicotoxin **HTX** that will be discussed more fully in Chapter 5 of this thesis. Meanwhile, the initial successful enantioselective *aza*-Michael result was deemed worthy of further investigation for reaction of the lithium ion of amine **325** to a range of (*E*)-α,β-unsaturated *tert*-butyl ester substrates in order to delineate its scope and limitation.

4.5 Investigation of the Asymmetric *Aza*-Michael Reaction of *N*-(4-Methoxybenzyl)-1,1,1-trimethylsilanamide to α,β-Unsaturated *tert*-Butyl Ester Analogues

4.5.1 Synthesis of α,β-Unsaturated *tert*-Butyl Ester Analogues

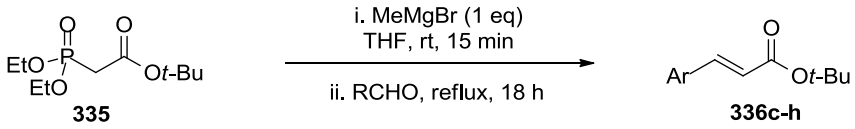
It was first necessary to prepare a range of aliphatic, aromatic and heteroaromatic (*E*)-α,β-unsaturated *tert*-butyl esters containing various functional groups. The desired compounds were either not available commercially or prohibitively expensive, so a reliable synthetic method using inexpensive reagents was sought. Many methods have been reported to give (*E*)-unsaturated alkenes such as the Julia olefination,[34-35] Peterson reaction,[36] and the Wittig reaction.[37-38] Recently lithium and magnesium in combination with nitrogen bases have been used for the HWE reaction of chiral aldehydes with phosphonate esters, giving good yields and (*E*)-selectivity.[39-40] Davies and co-workers have improved on this methodology by developing a simple protocol using MeMgBr as both a base and source of chelating metal for the HWE reaction of *tert*-butyl phophonate ester **335** with a range of aliphatic and aromatic aldehydes (Scheme 94).[41] The desired (*E*)-α,β-unsaturated *tert*-butyl esters **336** were reported to be isolated in excellent yields and with very high diastereoisomeric purities [(*E*):(*Z*) >98:2].

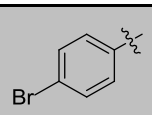
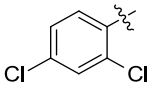
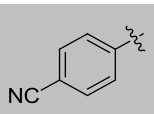
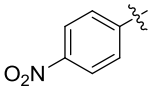
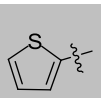
Scheme 94 *MeMgBr mediated HWE reaction*



This protocol seemed particularly suitable since phosphonate ester **335** was commercially available and a number of aldehyde substrates were available in the laboratory. Initially 4-bromobenzaldehyde was reacted with the anion of phosphonate ester **335** using the Davies protocol, giving the desired (*E*)-α,β-unsaturated *tert*-butyl ester in a yield of 72% (Table 11 entry 1). Therefore, five further aldehydes were reacted with **335** and the corresponding (*E*)-α,β-unsaturated *tert*-butyl esters isolated in acceptable yields (69-82%) and with excellent levels of diastereoselectivity (>97% *E*-isomer) (Table 11 entries 2-6).

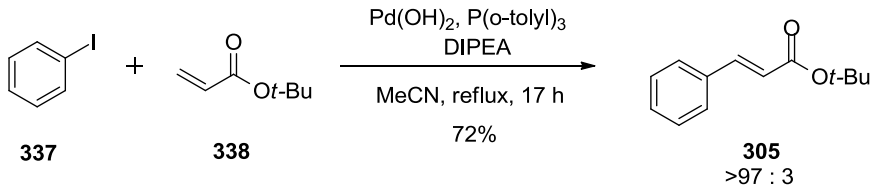
Table 11 *HWE synthesis of (E)-α,β-unsaturated esters*



Entry	*Ar-substituent*	*E*:*Z*	Yield (%)
1		>97 : 3	72
2		>97 : 3	78
3		>97 : 3	82
4		>97 : 3	69
5		>97 : 3	67
6		>97 : 3	65

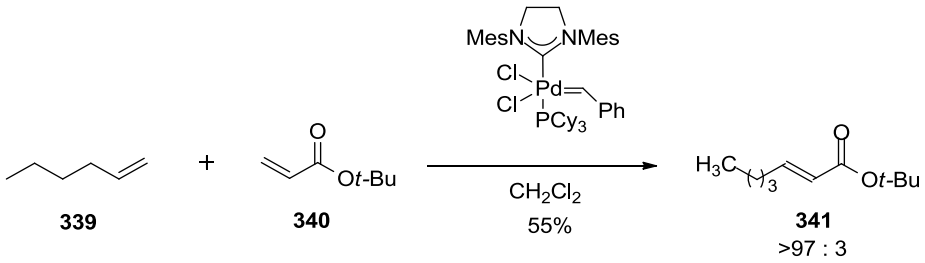
An alternative Suzuki-coupling methodology was used for the synthesis of *tert*-butyl cinnamate **305** involving treatment of iodobenzene **337** and *tert*-butyl acrylate **338** with $Pd(OH)_2$, P(o-tolyl)$_3$ and DIPEA in MeCN at reflux. Purification of the crude product by silica gel column chromatography gave a good yield of *E-tert*-butyl cinnamate **305** (Scheme 95).

Scheme 95 *Suzuki coupling for synthesis of **305***



α,β-Unsaturated ester **341** was efficiently synthesised by Grubbs olefin metathesis of hex-1-ene **339** and *tert*-butyl acrylate **340** that furnished its *E*-isomer in 55% yield after purification by silica gel chromatography (Scheme 96).

Scheme 96 *Grubbs olefin metathesis for the synthesis of* ***341***

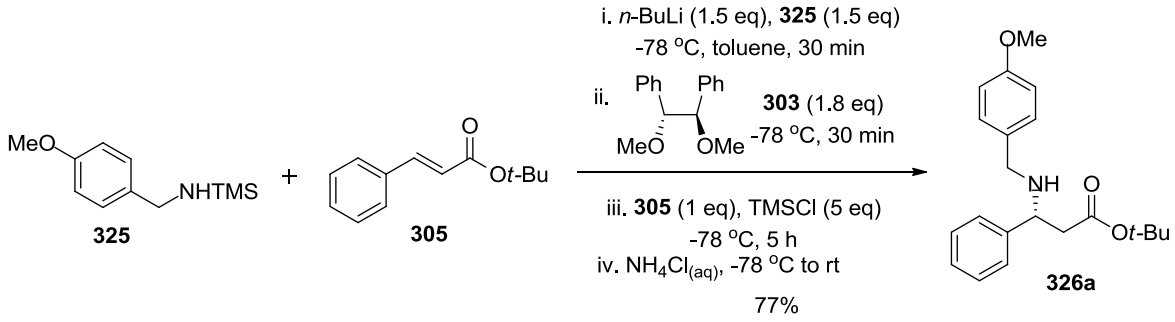


339 **340** **341**
>97 : 3

4.5.2 Asymmetric *aza*-Michael Addition of the Lithium Anion of *aza*-Nucleophile 325 to a Range of α,β-Unsaturated *tert*-Butyl Esters

The *aza*-Michael reaction of *N*-(4-methoxybenzyl)-1,1,1-trimethylsilanamine **325** was first trialed with *tert*-butyl cinnamate **305** using the same methodology employed for *bis*-α,β-unsaturated ester **4**, except for increased equivalents of amine **325**, *n*-BuLi and ligand **303** since *bis*-addition was not a problem with *mono*-α,β-unsaturated ester **305** (Scheme 97). Therefore a solution of amine **327** (1.5 eq) in toluene was added dropwise to a solution of *n*-BuLi in hexane/THF at -78 °C and the reaction was stirred for 30 minutes. Chiral ligand **303** (1.8 eq) was then dissolved in toluene and added dropwise and stirred for a further 30 minutes. A solution of α,β-unsaturated ester **305** (1 eq) and TMSCl (5 eq) in toluene was then added dropwise and the reaction mixture stirred for five hours before being quenched by the careful addition of saturated aqueous ammonium chloride. Purification of the crude reaction product by silica gel chromatography yielded the desired *N*-benzyl-β-amino ester **326a** in 77% yield and >97% e.e. (proved by chiral derivitisation and ^{1}H NMR spectroscopic analysis *vide infra*).

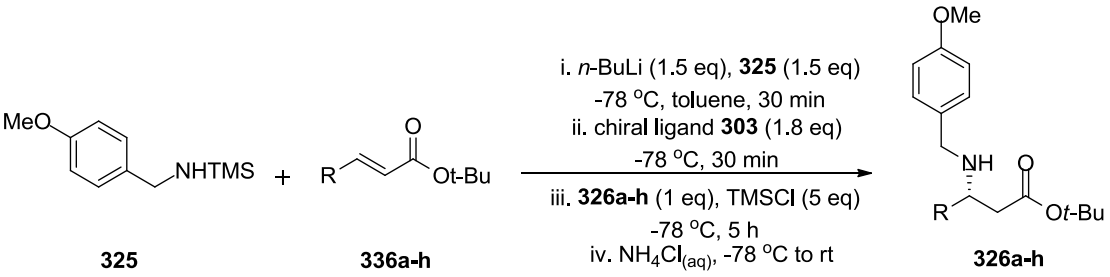
Scheme 97 *Aza-Michael reaction of N-(4-methoxybenzyl)-1,1,1-trimethylsilanamine* ***325***



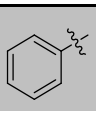
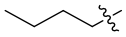
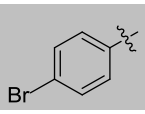
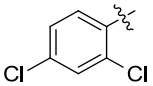
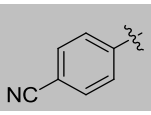
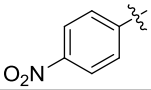
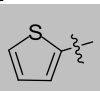
325 **305** **326a**

Delighted by this result, the scope of this *aza*-Michael methodology was further investigated by reacting the lithium anion of amine **325** with a range of β-substituted α,β-unsaturated ester analogues (Table 12). Entry 2 shows that the reaction was highly successful for aliphatic *R*-substituents, whilst entries 3-6 show a variety of aryl groups containing halo and electron withdrawing substituents were tolerated, giving good to excellent yields, except for the *para*-nitro derivative (entry 6) that gave a poorer yield of 40%. Entries 7 and 8 demonstrate that when heterocyclic rings were present in the β-position, good yields of the *aza*-Michael products could also be achieved. The generally high positive $[\alpha]_D$ values recorded for the *N*-benzyl-β-amino ester products suggested that these adducts had been formed with good enantiomeric excess.

Table 12 *Results of chiral ligand mediated aza-Michael reaction*



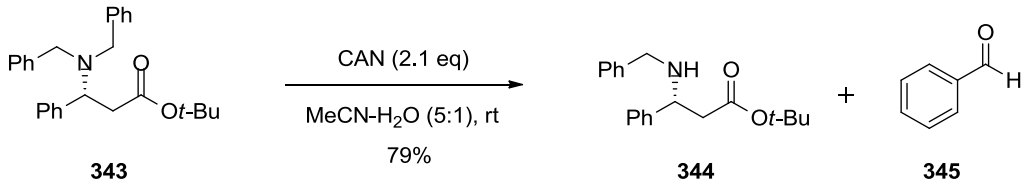
325 **336a-h** **326a-h**

Entry	*R*-substituent	Yield (%)	$[\alpha]_D$
1		77	+25.9
2		68	-3.5
3		79	+38.6
4		76	+24.1
5		62	+42.7
6		40	+35.7
7		86	+23.5
8		73	+57.3

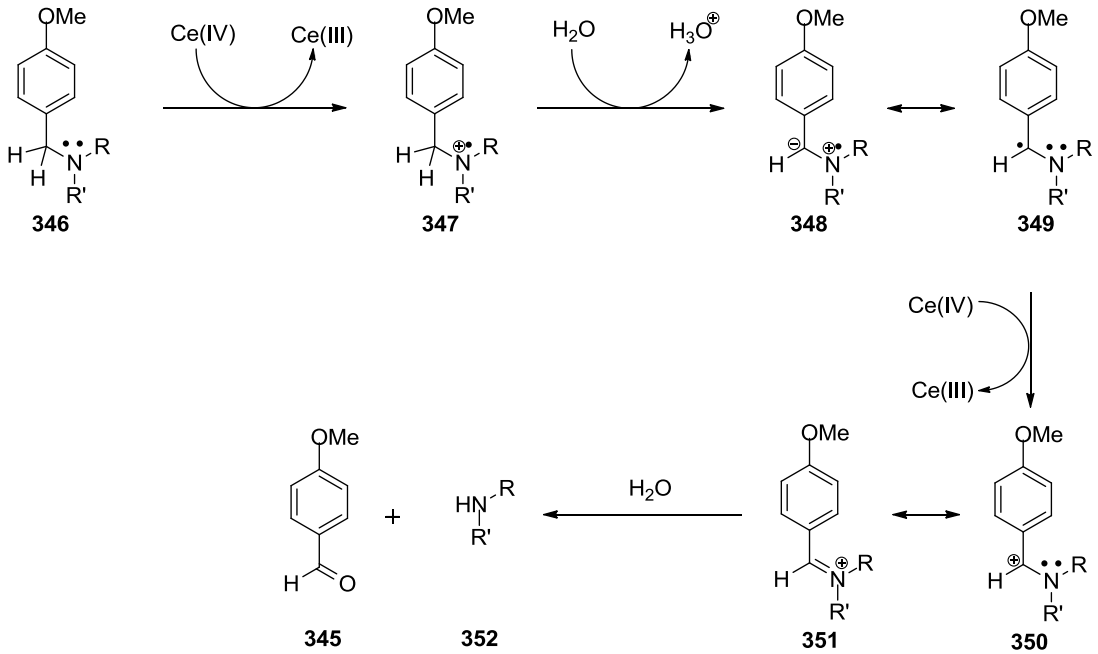
4.5.3 Oxidative CAN Deprotection of *aza*-Michael Addition Products

The oxidative deprotection of amine **326a** was trialed using standard CAN methodology previously reported by Davies and co-workers for the *N*-debenzylation of tertiary amines (Scheme 98),[42-43] as this would allow for the efficient orthogonal deprotection of amine functionality in the presence of functional groups sensitive to hydrogenolytic conditions. The mechanism of this oxidative debenzylation involves a Ce (IV) cation abstracting an electron from the lone-pair of the nitrogen to give radical cation **347**. The benzylic protons of **347** are acidic, and therefore one of them is removed by water to afford zwitterion **348**. Tautomerisation of anion **348** affords benzylic radical **349** that undergoes further Ce (IV) cation mediated oxidation to afford carbocation **350**, which tautomerises to iminium species **351**. Iminium cation **351** is then hydrolysed by water to afford *para*-methoxybenzaldehyde **345** and the deprotected amine **352** (Scheme 99).

Scheme 98 *Example of CAN oxidative N-debenzylation of tertiary amine*

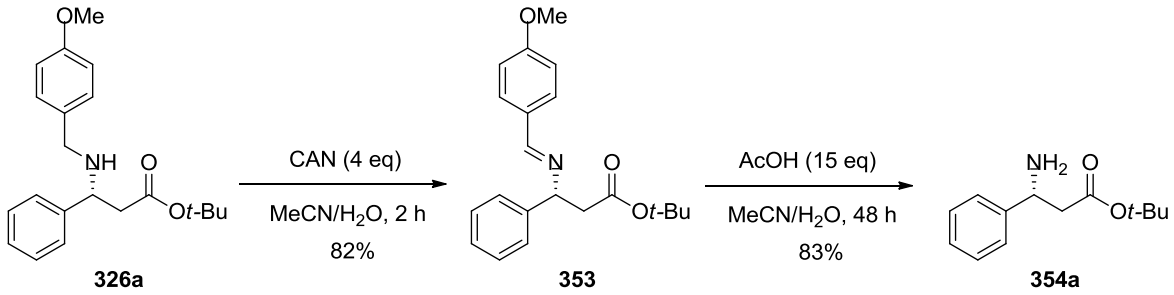


Scheme 99 *Mechanism of CAN oxidative deprotection*



When four molecular equivalents of CAN were added to a MeCN/H_2O (5:1) solution of protected amine **326a**, TLC showed that all of the starting material was consumed after just two hours, so the reaction was worked-up to afford a quantitative yield of imine **353** (Scheme 100). Imines are easily hydrolysed under acidic aqueous conditions, but *tert*-butyl esters are also susceptible to cleavage under mild acidic conditions. A number of trials were conducted varying the acid, solvent and reaction time to find conditions that would exclusively hydrolyse the imine functionality, thus maximising the yield of β-amino ester **354a**. It was found that stirring imine intermediate **353** in MeCN/H_2O (5:1) with 15 equivalents of acetic acid at room temperature for 48 hours gave the best results furnishing β-amino-*tert*-butyl ester **354a** in 83% yield.

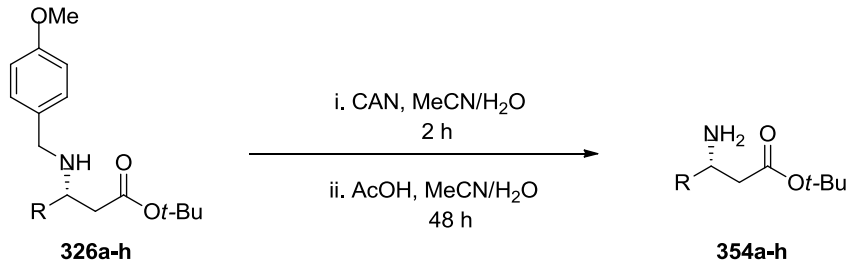
Scheme 100 *CAN oxidation followed by acidic hydrolysis for the deprotection of PMB protected amine* ***326a***



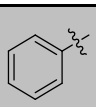
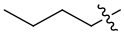
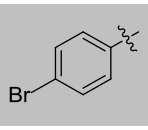
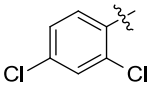
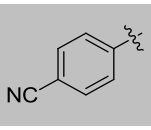
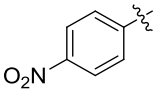
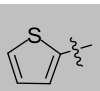
326a **353** **354a**

This oxidative deprotection methodology was then applied to the deprotection of the other *aza*-Michael products to give their corresponding β-amino ester products in average to good yields (Table 13). The lower yields reported in entries 3, 5 and 7 were due to competing hydrolysis of the *tert*-butyl ester in the imine cleavage step to afford their corresponding acids, which were found to be present in the aqueous washings by mass spectroscopic analysis. The enantiomeric excess of the resultant β-amino esters **354a-h** were confirmed as >97% e.e. *via* derivatisation with 2-formylphenyl boronic acid and enantiopure BINOL, followed by ^{1}H NMR spectroscopic analysis of the resultant diastereomeric imino-boronate ester complexes (*vide infra*).

Table 13 *CAN deprotection results for N-benzyl-β-amino ester* ***354a-h***



326a-h **354a-h**

Entry	*R*-substituent	Yield (%)	% e.e.[a]
1		68	>97
2		60	>97
3		56	>97
4		68	>97
5		51	>97
6		65	>97
7		36	>97
8		73	>97

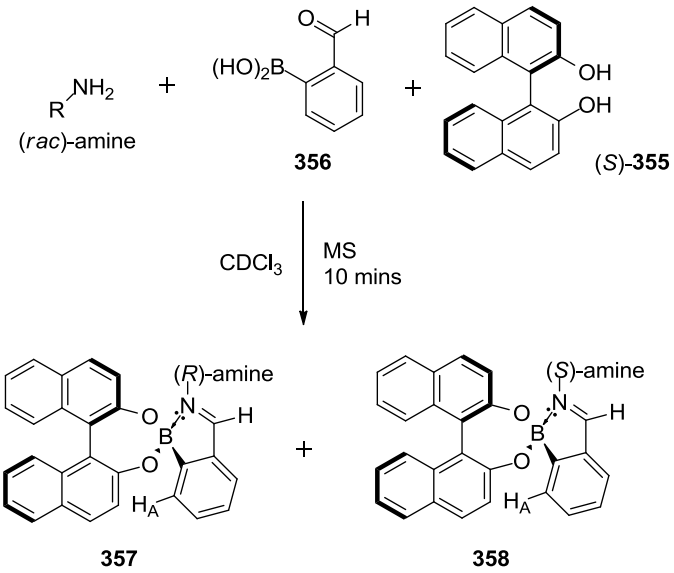
a. % e.e. determined by Bull and James chiral derivitisation NMR methodology *vide infra*[44-45]

4.5.4 Chiral Derivatisation of β-Amino Esters 354a-h to Determine their Enantiomeric Excess by ^{1}H NMR Spectroscopic Analysis

The enantiomeric excess of the β-amino esters **354a-h** were determined using a three-component NMR methodology developed by Bull, James and co-workers.[44-50] This

approach involves treatment of a chiral amine with an enantiopure BINOL, such as (*S*)-**355**, and 2-formylphenyl boronic acid **356** to afford a mixture of diastereomeric imino-boronate esters **357** and **358** (Scheme 101).[45,48] It has been found that the enantiomers of primary amines generally afford diastereomeric imino-boronate esters whose resonances are normally well resolved in their ^{1}H NMR spectra.[45] Since no kinetic resolution occurs in the derivatisation process, comparison of the integrals of multiple diastereomeric resonances allowed simple accurate determination of the enantiomeric excess of the parent amine.

Scheme 101 *Three-component derivatisation of chiral amines to afford mixtures of diastereoisomeric imino-boronate esters*



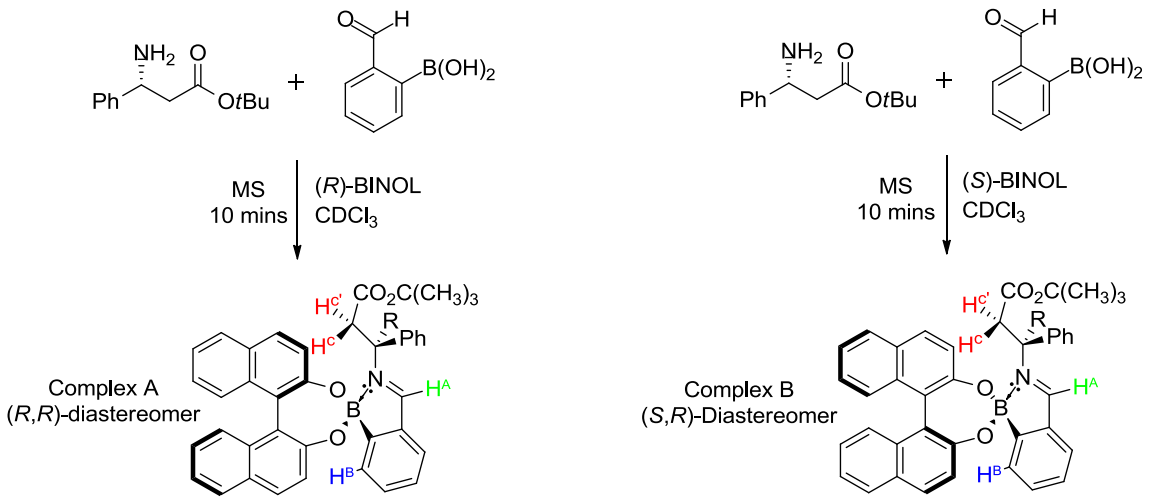
The scope of this chiral derivatisation methodology has been extended to determine the enantiomeric excess of C_2-symmetric diamines and *O*-silyl-1,2-amino alcohols.[46,49] It has also been demonstrated that other 1,2-, 1,3- and 1,4-chiral diols can be used as chiral auxiliaries for imino-boronate ester formation,[47] permitting the optimisation of the three-component derivitisation protocol for specific amines.[49] The enantiomeric excess of chiral diols can also be determined by using an enantiopure amine in the three-component reaction.[44,47,50]

Therefore, the enantiomeric excesses of β-amino ester products **354a-h** were determined using this three-component methodology. For example, β-amino-ester **354a** was reacted with enantiopure (*R*)-BINOL (*R*)-**355** and 2-formylphenyl boronic acid **356** to afford imino-boronate complex A (Scheme 102) and its ^{1}H NMR spectrum acquired (Figure 31i). This derivatisation reaction was then repeated using (*S*)-BINOL (*S*)-**355**, to afford the

diastereomeric imino-boronate complex B (Scheme 102) whose ^{1}H NMR spectrum was also acquired (Figure 31ii).

On comparison of the ^{1}H NMR spectra of complexes A and B, the resonances for the H^{A}, H^{B}, H^{C}, $H^{C'}$ and the *tert*-butyl protons of each diastereomer showed significant chemical shift differences (0.1-0.4 ppm). Thus showing clearly that one diastereomer is present in each spectrum. Integration of the *tert*-butyl proton resonances at 0.85 ppm and 1.15 ppm for complexes A and B easily allowed the diastereomeric excess of the parent β-amino ester **354a** to be determined as >97% e.e. The enantiomeric excess of the other β-amino ester products **354b-h** were all determined in the same manner, revealing that they had all been synthesised in >97% e.e.

Scheme 102 *Chiral derivitisation of β-amino ester product* ***354a***



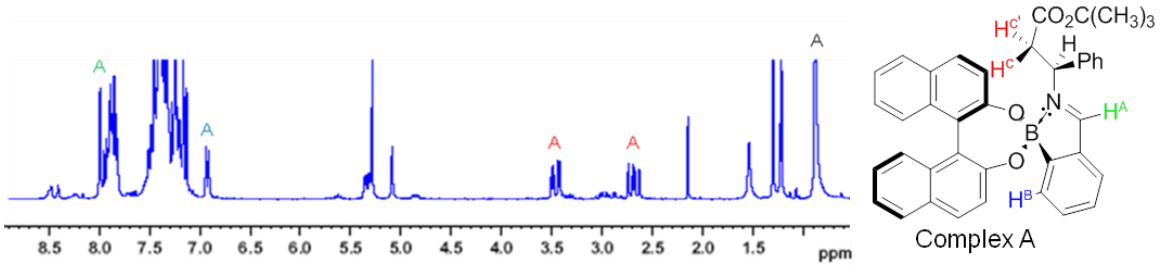
4.5.5 Assignment of the Absolute Configuration of β-Amino Ester Products

The absolute configuration of **354a** was assigned by comparison of the ^{1}H NMR spectra of the imino-boronate complexes A and B with the ^{1}H NMR spectrum reported previously for diastereomeric imino-boronate complexes of known configuration (Figure 31iii).[45] Therefore, the chiral ligand **303** mediated *aza*-Michael reaction was shown to afford the (*R*)-*N*-benzyl-β-amino ester product **326a**, which on deprotection by treatment with CAN and AcOH/H_2O furnished (*R*)-β-amino ester **354a** in >97% e.e. This conclusion was confirmed by comparison of the sign and magnitude of the specific rotation of (*R*)-*N*-benzyl-β-amino ester **326a** $[\alpha]_D$ +20.0 (*c* 1.2, $CHCl_3$), with the literature value for (*R*)-*N*-benzyl-β-amino ester **326a** that has been reported as $[\alpha]_D^{23}$ +19.7 (*c* 0.96, $CHCl_3$).[51]

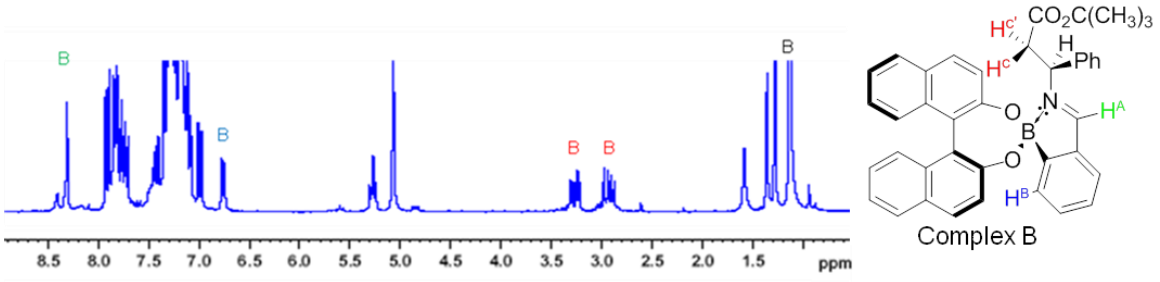
This is the same sense of asymmetric induction found by Tomioka and co-workers when they used the same chiral diether ligand **303** for the *aza*-Michael reaction of *N*-benzyl-1,1,1-trimethylsilanamine **304** to α,β-unsaturated *tert*-butyl esters (Section 4.2.5).[27]

Figure 31 *^{1}H NMR spectra of imino-boronate complexes A, B and literature spectrum of rac-complex for comparison*

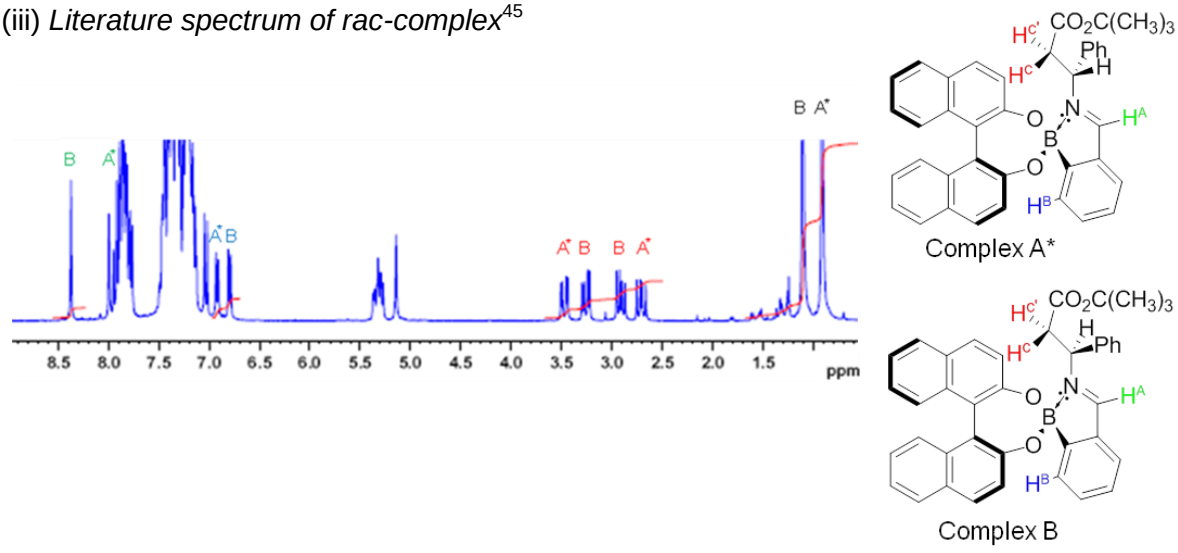
(i) *Complex A*



(ii) *Complex B*



(iii) *Literature spectrum of rac-complex*[45]



A*=resonances due to (*S,S*)-complex which is an enantiomer of Complex A and therefore identical by ^{1}H NMR

4.6 Conclusion

A key β-amino ester intermediate **5** for the synthesis of Histrionicotoxin **HTX** has been synthesised *via* highly stereoselective *aza*-Michael addition of the chiral lithium anion of *N*-trimethylsilyl-*para*-methoxybenzylamine **325** to *bis*-α,β-unsaturated *tert*-butyl ester **4** mediated by an enantiopure chiral diether ligand **303**. This methodology has then been applied to a range of α,β-unsaturated *tert*-butyl esters. Contrary to earlier reports, amide **325** was found to be a good nucleophile for this *aza*-Michael reaction, when Tomioka's optimised *aza*-Michael methodology was used. These *aza*-anion conjugate addition reactions proved to be highly stereoselective, affording *N*-benzyl-β-amino ester products **326a-h** in >97% e.e. with a variety of aliphatic, aromatic and heteroaromatic α,β-unsaturated *tert*-butyl esters. Each *N*-benzyl-β-amino ester product was oxidatively deprotected with CAN to yield β-amino ester products **354a-h**, after treatment of their imine intermediates with AcOH. Since these CAN oxidative conditions enable the deprotection of PMB amines, this methodology provides a useful alternative for preparing β-amino esters containing functionality that is sensitive to hydrogenolytic conditions.

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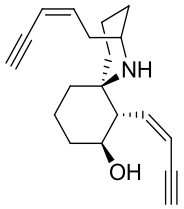
Chapter 5

5.1 Introduction

With efficient *aza*-Michael addition methodology having been developed for the asymmetric synthesis of β-amino esters, the research focus turned towards the synthesis of Histrionicotoxin **HTX** (Figure 32). **HTX** has an impressive spiro[5.5]undecane ring system with four stereocentres and two *cis*-enyne side-chains that has been shown to be a potent non-competitive nicotinic acetylcholine receptor (nAChR) antagonist.[1] Previously a number of syntheses of **HTX** have been reported that suffer from low overall yields and involve many steps. The approach investigated proposed to rapidly construct the carbon backbone of **HTX** and introduce the nitrogen functionality enantioselectivity using the *aza*-Michael methodology described in Chapter 4. Attempts would then be carried out to achieve spirocyclisation applying ester enolate-imine methodology recently developed by Bull and co-workers.[2]

Figure 32 *Structure of the frog alkaloid Histrionicotoxin*



HTX

5.2 Biological Activity of Histrionicotoxin

HTX was first isolated by Daly in 1971 from skin extractions of the Columbian poison arrow frog *Dendrobates histrionicus.*[3] Over the following years a further 14 spirocyclic alkaloids have been isolated from frogs of the family *Dendrobatidae*. The compounds of this family share the unique core spiropiperidine structure and, with the exception of the three deoxygenated members, vary only in the length and degree of saturation present in their two side chains. **HTX** and other spirocyclic alkaloids have been found to be potent non-competitive nAChR antagonists. nAChRs are a class of cholinergic receptors activated by acetylcholine (ACh) as well as other neurotransmitters that were originally characterised by the agonistic effect that nicotine exerts on them. When ACh binds to a nAChR an associated ion channel is opened and an influx of ions elicits a response (Figure 33).

Figure 33 *The sequence of events at a typical cholinergic synapse*[4]

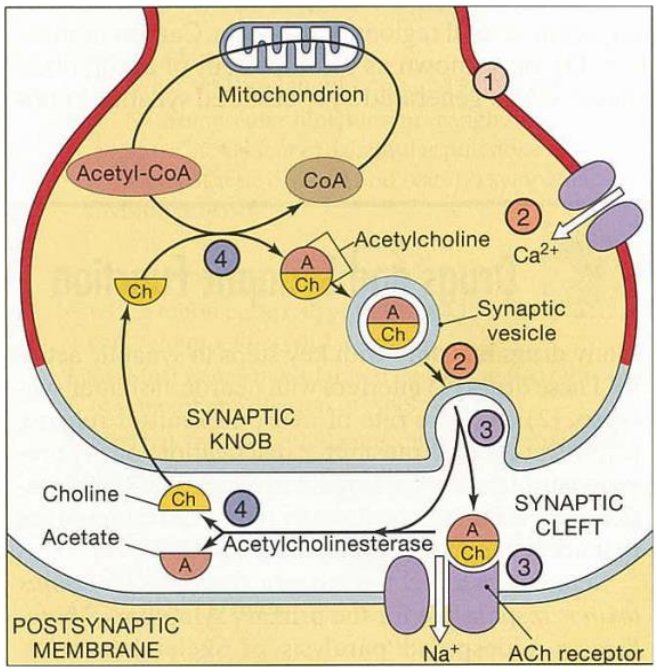
Step 1

An arriving action potential depolarises the synaptic knob.

Step 2

Calcium ions enter the cytoplasm of the synaptic knob.

ACh is released through exocytosis of neurotransmitter vesicles.



Step 3

ACh diffuses across the synaptic cleft and binds to receptors on postsynaptic membrane.

Chemically regulated sodium channels on the postsynaptic surface are activated, producing a graded depolarization.

ACh release ceases as calcium ions are removed from the cytoplasm of synaptic knob.

Step 4

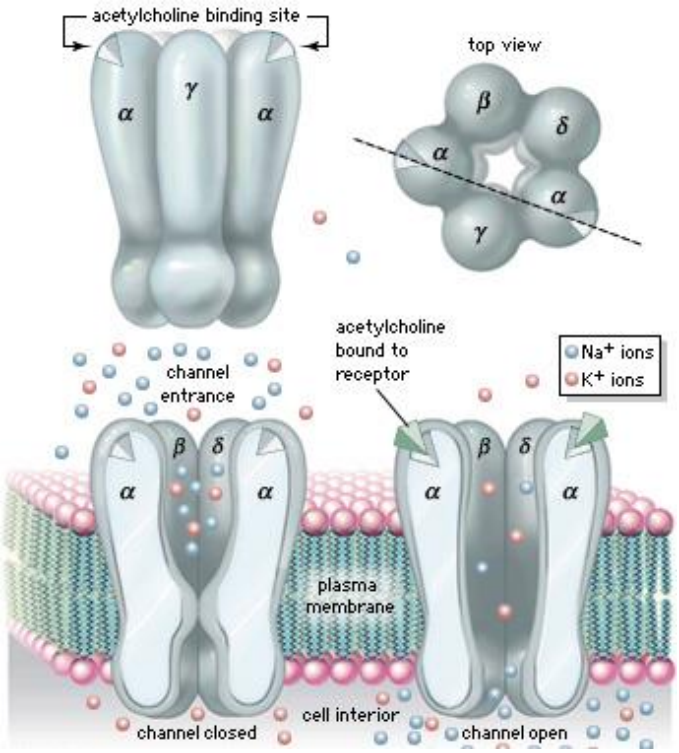
The depolarisation ends as ACh is hydrolysed into acetate and choline by acetylcholinesterase.

The synaptic knob reabsorbs choline from the synaptic cleft and uses it to resynthesize ACh.

These nAChRs are found at the nerve-nerve synapses and nerve-muscle synapses of the peripheral nervous system, the ganglion and in the central nervous system where, except for a few exceptions, they appear at the post-synaptic membrane. The structure of the nAChR of the *Torpedo californica* electic ray has been successfully characterised.[5] It is a

protein complex made up of five subunits. The five subunits (β, γ, δ and two α units) form a cylindrical shape which traverses the cell membrane with the centre of the cylinder acting as a gated ion channel for Na^+, K^+ or Ca^{2+} (Figure 34). The nAChRs found in the human body differ according to the structure of their α- and β-subunits and show specific binding affinities, which can be probed by small molecule ligand binding studies.[6]

Figure 34 *Nicotinic acetylcholine receptor image*[7]



Results of ligand binding studies on *Torpedo californica* electroplax, rat pheochromocytoma PC12 cells and frog nerve-muscle preperations have demonstrated the potent antagonistic effects of **HTX**.[1,8-10] The *Torpedo californica* electroplax contain a high density of nAChRs that are analogous to the muscular nAChRs of other vertebrates. **HTX** does not inhibit the binding of acetyl choline, but instead augments its affinity for the receptor site at **HTX** concentrations of 2 μM and 8 μM.[8] Contrastingly, **HTX** has a binding constant[1] of 0.63 μM for the binding inhibition of radio labelled perhydrohistrionicotoxin **361** (a compound that is known to bind at an allosteric site on the nAChR).[9] Therefore, it can be concluded that, for muscular nAChRs, **HTX** acts as a non-competitive antagonist.

[1] The binding constant (Ki) $\mathrm{Ki} = {IC50}/{1 + [S]/Km}$

For the rat pheochromocytoma PC12 cells that have nAChRs representative of a ganglion nAChRs, **HTX** also has a strong non-competitive antagonistic effect with an IC_{50} of 4.3 μM recorded for an assay where the $^{22}Na^{+}$ influx was measured with a 2 mM concentration of the nAChR agonist carbamylcholine.[1] Daly and co-workers used frog nerve-muscle preparations to test the effect of spirocyclic alkaloids on nerve-muscle preparations.[9] They showed that a **HTX** concentration of 5 μM blocked the normal nerve evoked muscle twitch, with repetitive stimulation resulting in the decreased rate and strength of muscle action potentials. From these results they concluded that **HTX** was not only behaving as a non-competitive nAChR antagonist, but also acting on the sodium and potassium ion channels. They speculated that the site of **HTX** binding could in fact be at the lipid-protein interface since **HTX** was found to have a high affinity for an intrinsic membrane protein.

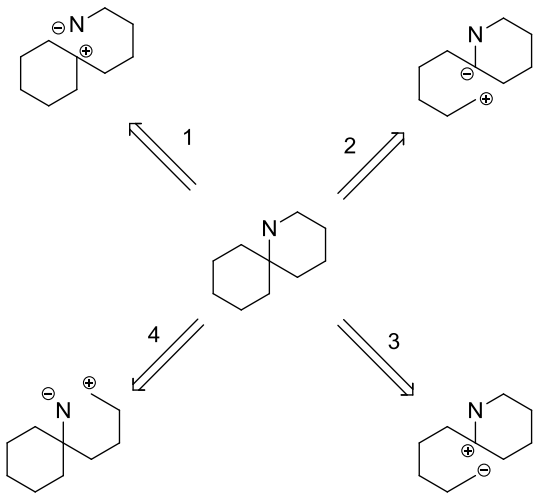
Central nervous system research opportunity

It is known that there are a number of subtypes of neuronal nAChRs, these nAChRs are associated with Ca^{2+} transfer as well as Na^{+} and K^{+} permeability. Changing the concentration of Ca^{2+} in the cell affects a diverse range of signalling pathways. Modulation of the neurotransmitter release by nAChRs has been well documented and in some cases particular subtypes of nAChRs have been connected to the release of particular neurotransmitters.[11] There is a pressing need for small molecules that have a high affinity and selectivity for these receptors that can be used as biological tools to selectively interrogate these complex systems. It was hoped that in collaboration with the Wonnacott group, testing **HTX** and other synthetic analogues for selective antagonistic behaviour with neuronal nAChRs would enable new selective antagonists to be identified that could further the understanding of this important class of receptor.

5.3 Previous Syntheses of Histrionicotoxin and Related [5.5] Spiro-2-Piperidines

Over the last four decades a considerable amount of synthetic interest has focused on the HTX family of alkaloids. The first synthesis of *rac*-**HTX** was reported by Kishi and co-workers in 1985 and since then seven further total syntheses have been reported, as well as a number of formal syntheses.[12-19] This mini review will be divided into four sections, each one focusing on selected ring disconnections illustrated below (Scheme 103), and will include syntheses of **HTX** and other members of the HTX family of alkaloids to showcase the chemistry that has been developed in this area.

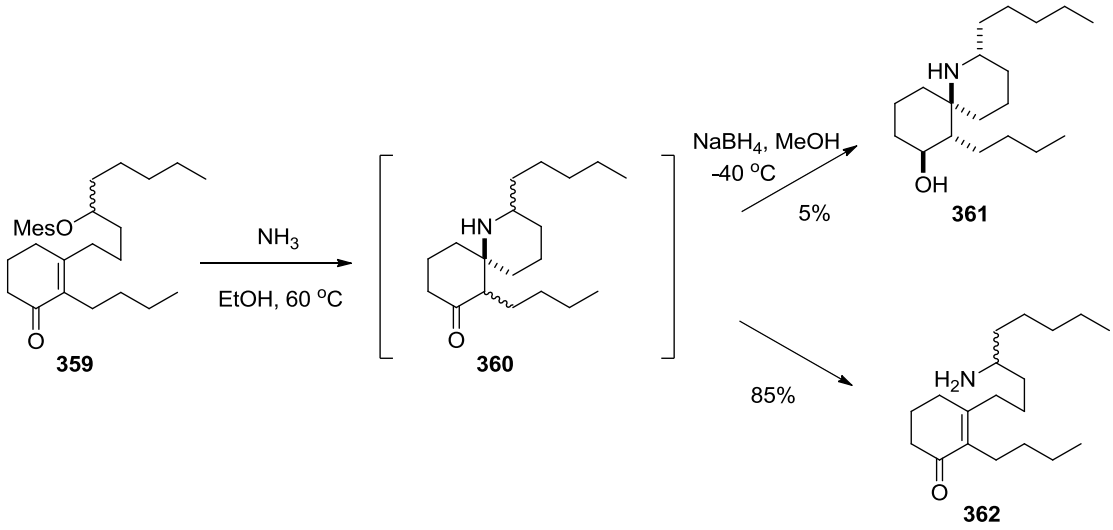
Scheme 103 *Potential disconnections for the synthesis of the spirocyclic core of* ***HTX***



5.3.1 Disconnection 1: Conjugate addition and the intramolecular ene reaction of amines

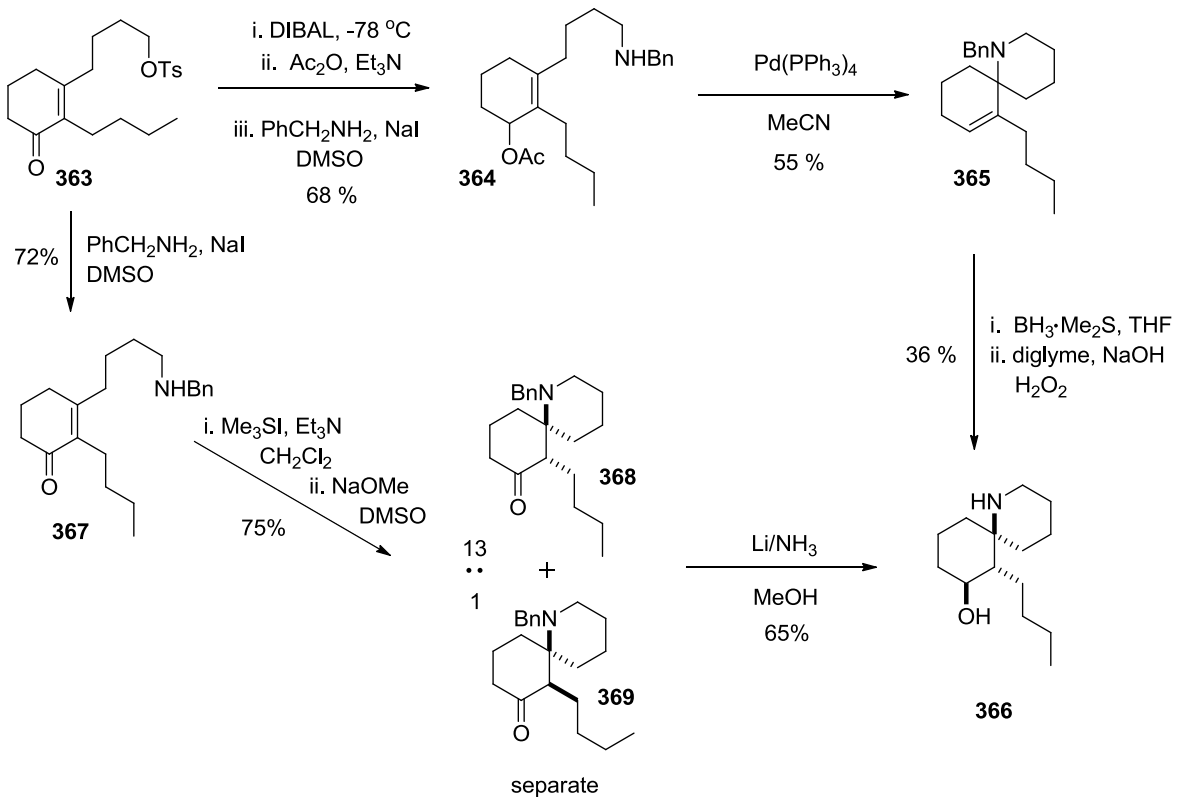
In 1976, Corey and co-workers attempted to employ an *aza*-Michael reaction, to convert α,β-unsaturated ketone intermediate **359** to *rac*-**361** (Scheme 104).[20] They initially attempted the reaction in a saturated aqueous ammonia solution, but could only isolate amine **362** in 85% yield despite some spectroscopic data indicating the presence of the target cyclised product **360**. By adding sodium borohydride to the reaction they managed to generate a complex mixture of products, from which it was possible to isolate a 5% yield of *rac*-**361**.

Scheme 104 *Corey synthesis of rac-****361***



Almost a decade later the Carruthers and Godleski groups simultaneously published syntheses of *rac*-depentyl-perhydrohistrionicotoxin **366** (Scheme 105). The Carruthers group prepared the protected amine **364** in good yield that underwent spirocyclisation in the presence of a catalytic amount of $Pd(PPh_3)_4$ in good yield.[21] To complete their synthesis they hoped to perform stereoselective hydroboration/oxidation reactions whose regioselectivity would be controlled by the proximal nitrogen. Unfortunately, they could only achieve an epimeric mixture of alcohols, but after isolation of the required diastereomer it was deprotected to give **366** in an overall yield of 13%. The Godleski group began their synthesis from the same starting material **363**, but then chose to install benzylamine and spirocyclise *via* an intramolecular *aza*-Michael reaction, before reduction of the ketone functionality.[22] The key spirocyclisation reaction gave a 13:1 ratio of diastereomers in favour of the desired product **368**, which was reduced to *rac*-**366** to complete the synthesis in a good overall 33% yield.

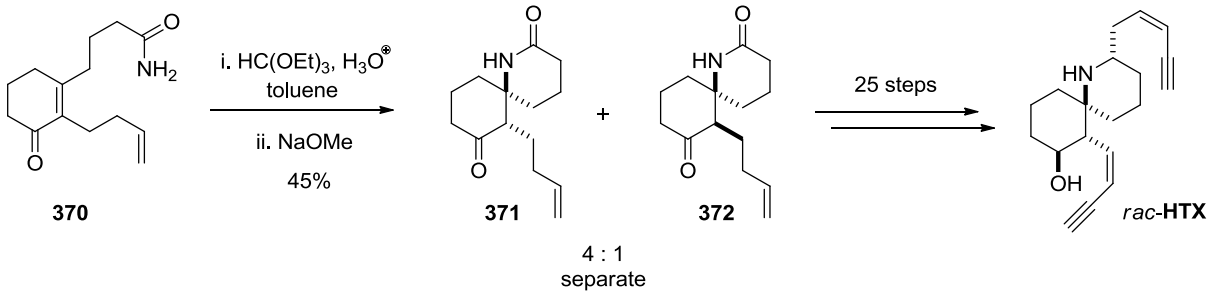
Scheme 105 *Carruthers and Godleski syntheses of rac-**366***



Kishi and co-workers built on these results to develop the first total synthesis of *rac*-**HTX** (Scheme 106).[12] The precursor **370** was first synthesised in 45% yield over four steps

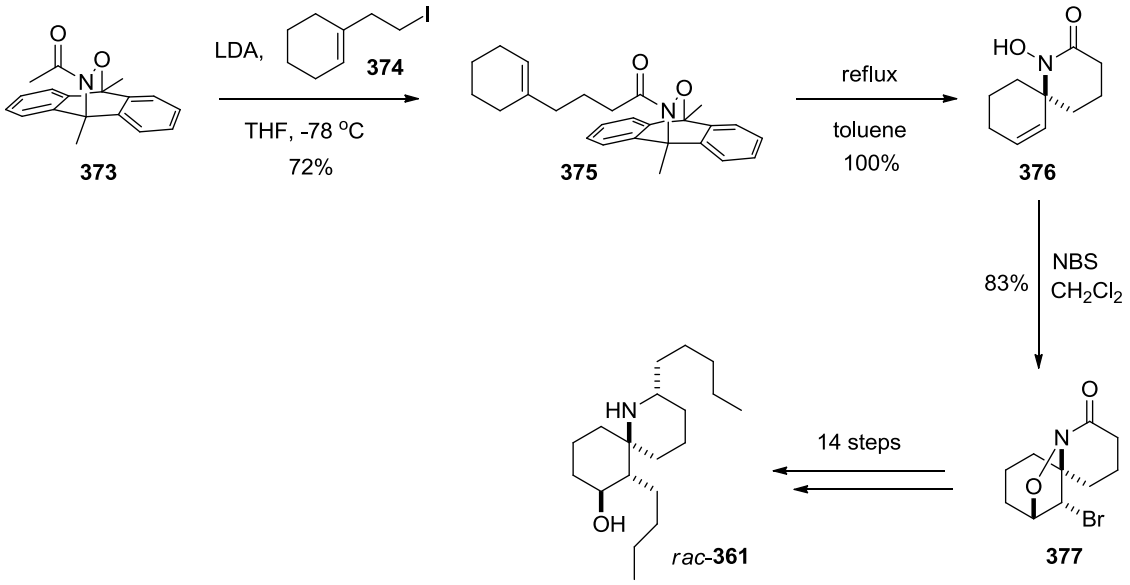
and then an intramolecular *aza*-Michael reaction carried out to afford diastereomers **371** and **372** in a 1:2 d.r. Fortunately, this epimeric mixture could be equilibrated with sodium methoxide to give a 4:1 d.r. in favour of the desired diastereomer **371**. The diastereomers were then separated by chromatography and **371** was elaborated to *rac*-**HTX** over an interminable 25 steps, with an overall yield of 0.02%!

Scheme 106 *Kishi first total synthesis of rac-**HTX***



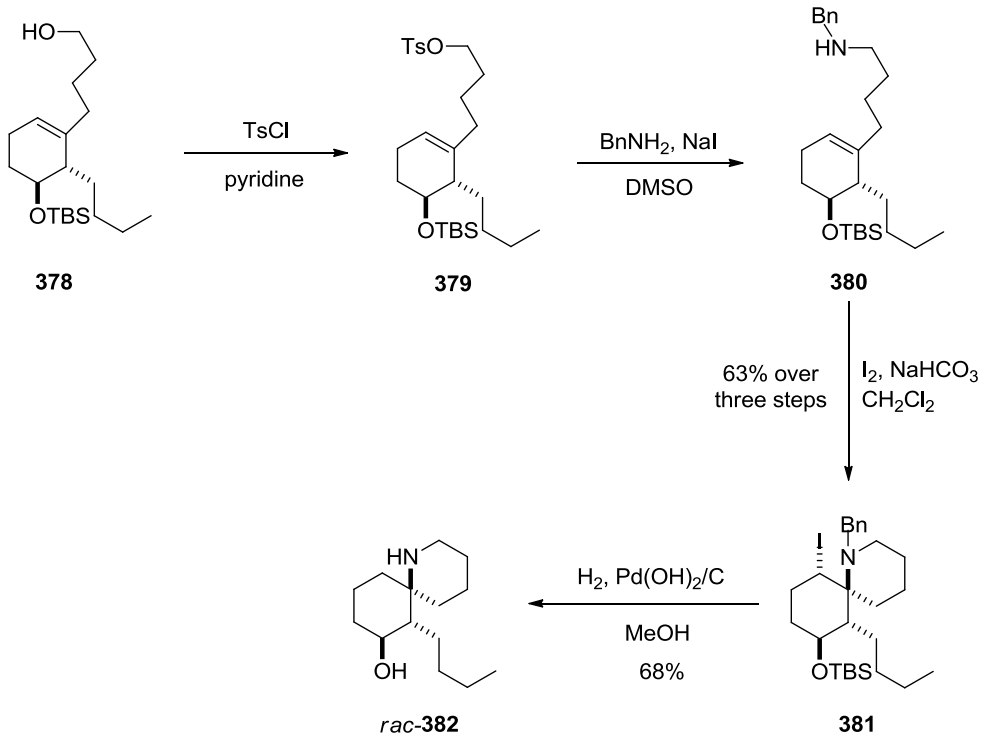
Keck and co-workers developed a novel synthesis involving an intramolecular ene reaction to generate the spiro-2-piperidine core for their synthesis of *rac*-**361** (Scheme 107).[23] Compound **373** was initially alkylated with 1-(2-iodoethyl)cyclohex-1-ene **374**. Thermolysis of product **375** triggered a *retro*-Diels-Alder reaction, and the resulting *N*-acylnitroso compound spontaneously underwent an intramolecular ene reaction to give *N*-hydroxy-lactam **376**. The *N*-hydroxy group was then used to install the C(8) hydroxyl group stereoselectively by intramolecular attack on a bromonium species giving **377**, which could be elaborated to *rac*-**361** over 14 steps.

Scheme 107 *Keck formal synthesis of rac-**361***



Tanner subsequently developed iodine promoted spirocyclisation methodology (Scheme 108).[24-25] The primary hydroxyl group of **378** was converted into a benzylamine, with addition of iodine to forming an iodonium ion that reacted intramolecularly with the amino group to afford spirocycle **381** that was debenzylated and dehalogenated *via* hydrogenolysis to the target spirocycle *rac*-**382**.

Scheme 108 *Tanner iodine promoted spirocyclisation methodology rac-**382***



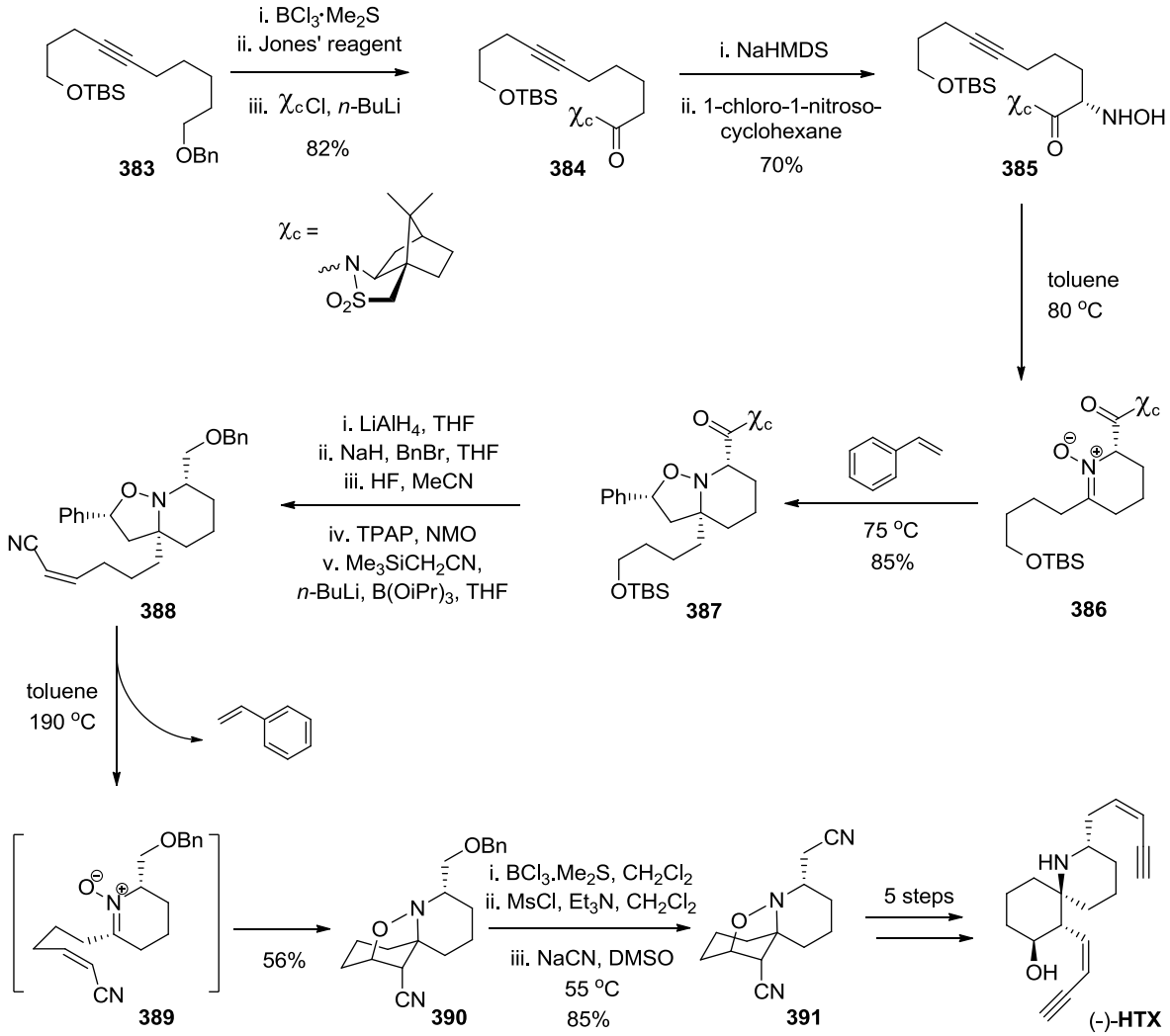
378 **379** **380** **381** *rac*-**382**

5.3.2 Disconnection 2: 1,3-Dipolar Cycloaddition Approach

In 1999 the Holmes group published an enantioselective route to (-)-**HTX** with an intramolecular [3+2] nitrone cycloaddition reaction as the key spirocyclisation step (Scheme 109).[14] Earlier work by other groups had shown that this reaction could be used to prepare spiropiperidines,[26-28] but only if the alkene substituents were not too sterically demanding. By employing **388** as the substrate that contains an alkene substituted with an electron withdrawing nitrile group, Holmes overcame these steric demands, allowing further elaboration of the nitrile functionality to form the target compound. (-)-**HTX** was synthesised by this method in an extended 20 steps, but in an acceptable overall yield of 16%. The stereochemistry was introduced during the [3+2] cylisation reaction using a chiral auxiliary to mediate reaction with 1-chloro-1-nitrosocyclohexane to form hydroxylamine **386** as a single isomer. They went on to apply the methodology to the

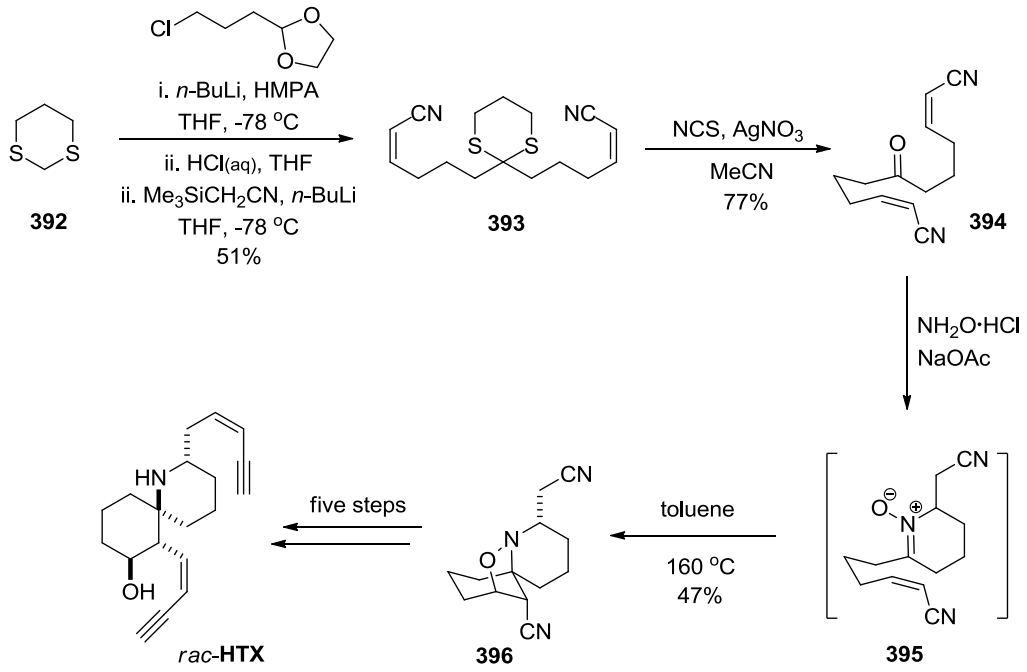
synthesis of the other enantiomer of the alkaloid, (+)-**HTX**, by using an alternative chiral auxiliary and protecting group strategy.[16]

Scheme 109 *Holmes total synthesis of (-)-**HTX***



Soon after Holmes' first total synthesis, Stockman and co-workers published a formal synthesis of *rac*-**HTX** that involved the synthesis of a C_2-symmetric acyclic precursor **393** (Scheme 110).[15] **393** was then reacted with hydroxylamine and heated in toluene in a sealed tube to give the spirocyclic structure **396**. This method was an improvement on the Holmes method since it used a more readily available precursor and was only eleven steps long.

Scheme 110 *Stockman total synthesis of rac-**HTX***

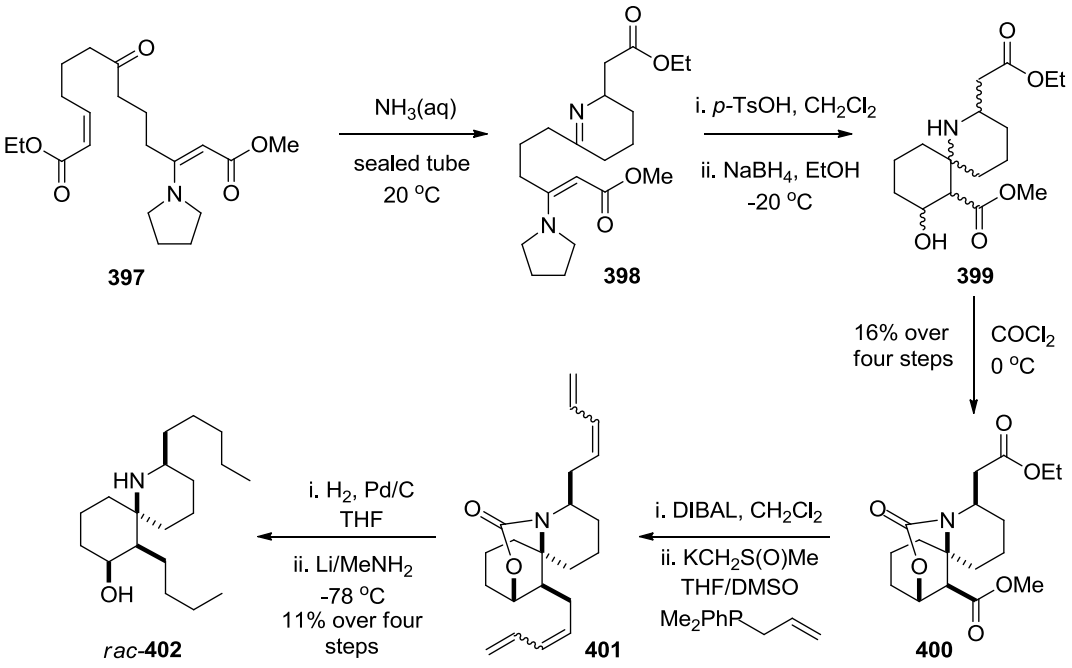


Stockman later published a total synthesis of *rac*-**HTX** in collaboration with the Fuchs group.[17] This used the spirocyclic ring forming methodology, but built the precursor *via* a more efficient route, elaborating **396** using alternative Wittig-based chemistry.

5.3.3 Disconnection 3: Mannich-type Reactions of Imines and Acyliminium Ions

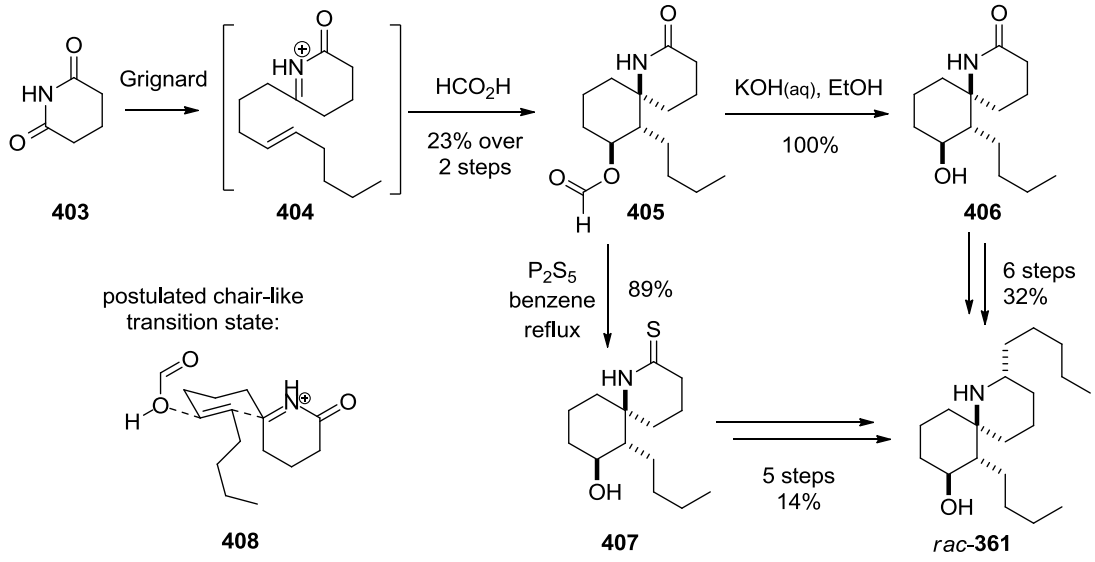
The potential of the Mannich reaction for spirocyclisation was first explored by Corey in his total synthesis of the unnatural HTX analogue *rac*-epi-2,7-perhydrohistrionicotoxin *rac*-**402** (Scheme 111).[29] Starting from bromocyclopentene they constructed the carbon backbone to give **397**. Condensation with liquid ammonia in a sealed tube with concomitant *aza*-Michael addition gave imine **398**. The key enamine-Mannich reaction was promoted by *p*-TsOH and the crude product was immediately reduced to yield **399** as a complex mixture of diastereomers. The required epimer was isolated from this mixture of diastereomers by reaction with phosgene to form a cyclic carbamate **400** and then elaborated over four steps to afford *rac*-**402**.

Scheme 111 *Corey synthesis of unnatural HTX analogue rac-**402***



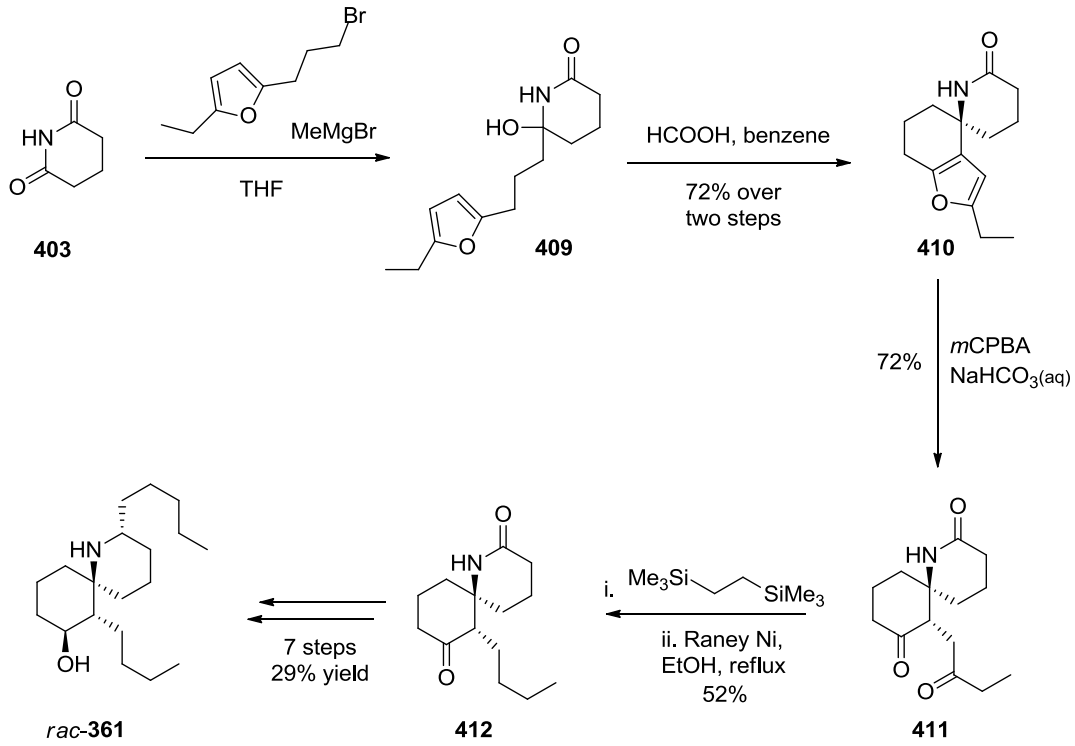
The Mannich reaction of of *N*-acyliminium ions was developed by both the Evans and Speckamp groups working on glutarimide **403** (Scheme 112).[30-31] Both groups began with a Grignard addition to glutarimide **403** forming an *N*-acyliminium ion intermediate **404** that underwent olefin cyclisation in the presence of formic acid. Spirocycle **405** was then elaborated to the desired product by different methods to arrive at the target molecule. Speckamp later rationalised the stereoselectivity of the cyclisation reaction by postulating a chair-like transition state **408** in which the butyl group preferentially adopts an equatorial position.[32]

Scheme 112 *Evans and Speckamp formal syntheses of rac-**361***



A similar strategy was adopted by the Tanis group to realise the formal synthesis of *rac*-**361** (Scheme 113).[33] A Grignard reaction with glutarimide provided **403**, contains a furan moiety that underwent an intramolecular cyclisation onto an *N*-acyliminium ion intermediate to give **410** with an impressive 72% yield over the two steps. The furan ring was then oxidatively cleaved to **411** using *m*-CPBA and the unstable ene-dione product was immediately reduced by using a Noyori kinetic ketalisation reaction followed by Raney nickel reduction to afford diketone **412**, which had been previously elaborated by Keck and Corey to the target compound *rac*-**361**.[23,34]

Scheme 113 *Tanis formal synthesis of rac-**361***

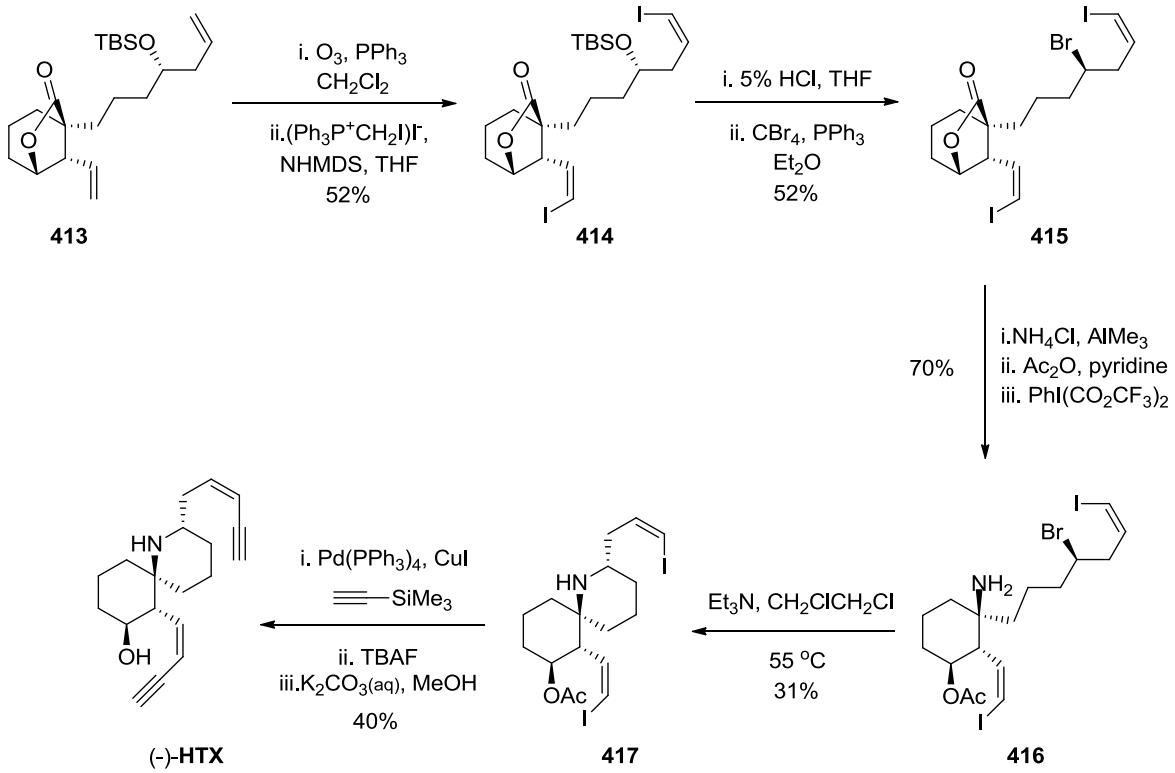


5.3.4 Disconnection 4: Intramolecular Nucleophilic Substitution Reactions

The Stork group have reported a novel synthesis of (-)-**HTX** where an intramolecular nucleophilic substitution reaction gave the key spirocyclic structure **417** (Scheme 114).[13,35] They initially synthesised intermediate **413** from enantiomerically pure starting materials and then subjected this molecule to ozonolysis and *Z*-selective Wittig methodology to yield the *bis*-(*Z*)-1-iodo-alkene **414**. The side-chains were subsequently elaborated to afford **415** and the amine functionality was then introduced by treating with trimethylaluminium-ammonium chloride followed by acetyl protection of the alcohol functionality giving an acetoxy amide, which underwent a Hoffman-like rearrangement on the addition of

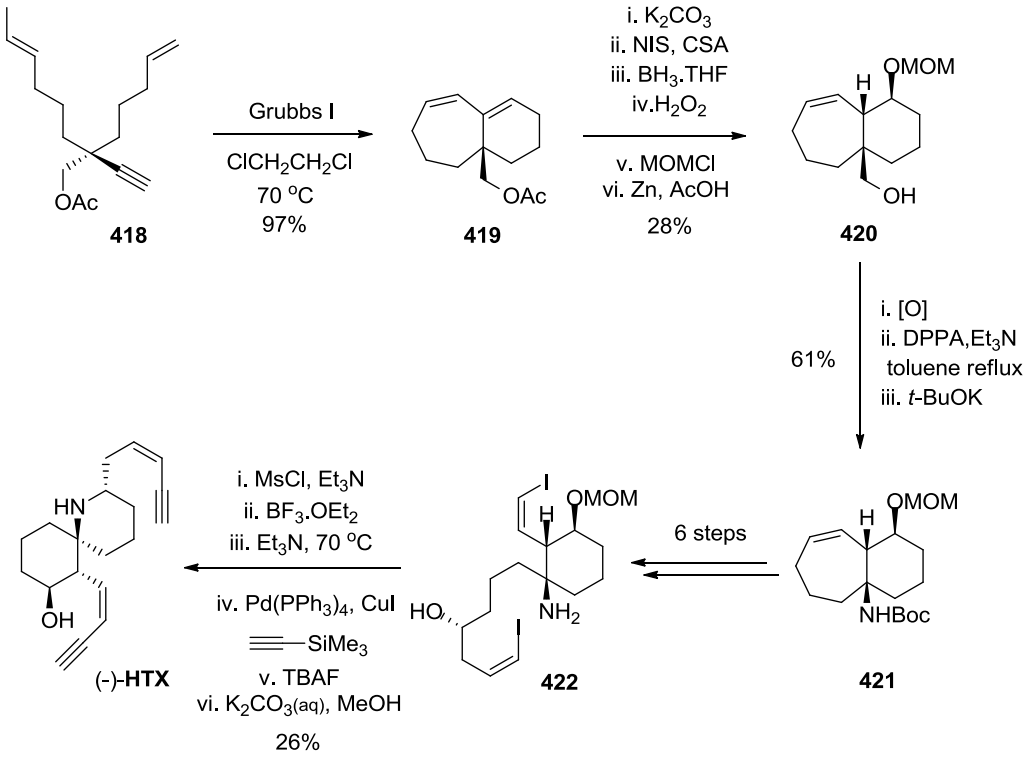
phenyliodonium bistrifluoroacetate to give **416**. Nucleophilic spirocyclisation then required heating of a 1,2-dichloroethane solution of bromide **417** and triethylamine at 55 °C. Coupling with (trimethylsilyl)-acetylene and deprotection of the spirocycle then gave (-)-**HTX** in an overall 1% yield over 14 steps.

Scheme 114 *Stork total synthesis of (-)-**HTX***



The Fukuyama group recently reported another synthesis of (-)-**HTX** using a nucleophilic substitution reaction to form the spirocyclic structure after an impressive range of chemistry to construct the precursor **422** (Scheme 115).[19] They subjected enantiomerically pure dienyne **418** to metathesis conditions to afford bicyclic structure **419** in high yield and enantioselectivity. The acetate group was then deprotected and used as a "chiral handle" to direct selective functionalisation of the alkene bonds. Primary alcohol **420** was oxidised and rearranged under Hoffman conditions to afford Boc-amide **421** that could be further elaborated to **422** before spirocyclisation by using a simple intramolecular nucleophilic substitution reaction. Functionalisation of the *Z*-alkene side-chains using the Stork methodology described above then gave (-)-**HTX** in 1% overall yield from advanced intermediate **418**.

Scheme 115 *Fukuyama total synthesis of (-)-**HTX***



5.3.5 Summary of Synthesis of HTX and Analogues

The synthesis of **HTX** and analogues has proved a testing challenge to a number of talented synthetic groups and a fertile ground for the development of methodology over the past four decades. Synthetic organic chemistry is an ever evolving discipline, so there is undoubtedly scope for new concise and novel strategies for the synthesis of **HTX** and analogues. If successful, this would enable libraries of spirocyclic compounds to be tested for selective antagonistic behaviour against specific subtypes of neuronal nAChRs that could lead to dramatic discoveries in the field of neurochemistry.

5.4 Synthetic Plan for the Total Synthesis of Histrionicotoxin

The initial plan for the synthesis of **HTX** can be simplified into five stages:

A. Construction of the acyclic bifunctional carbon backbone using standard organic chemistry (**2** to **424**).

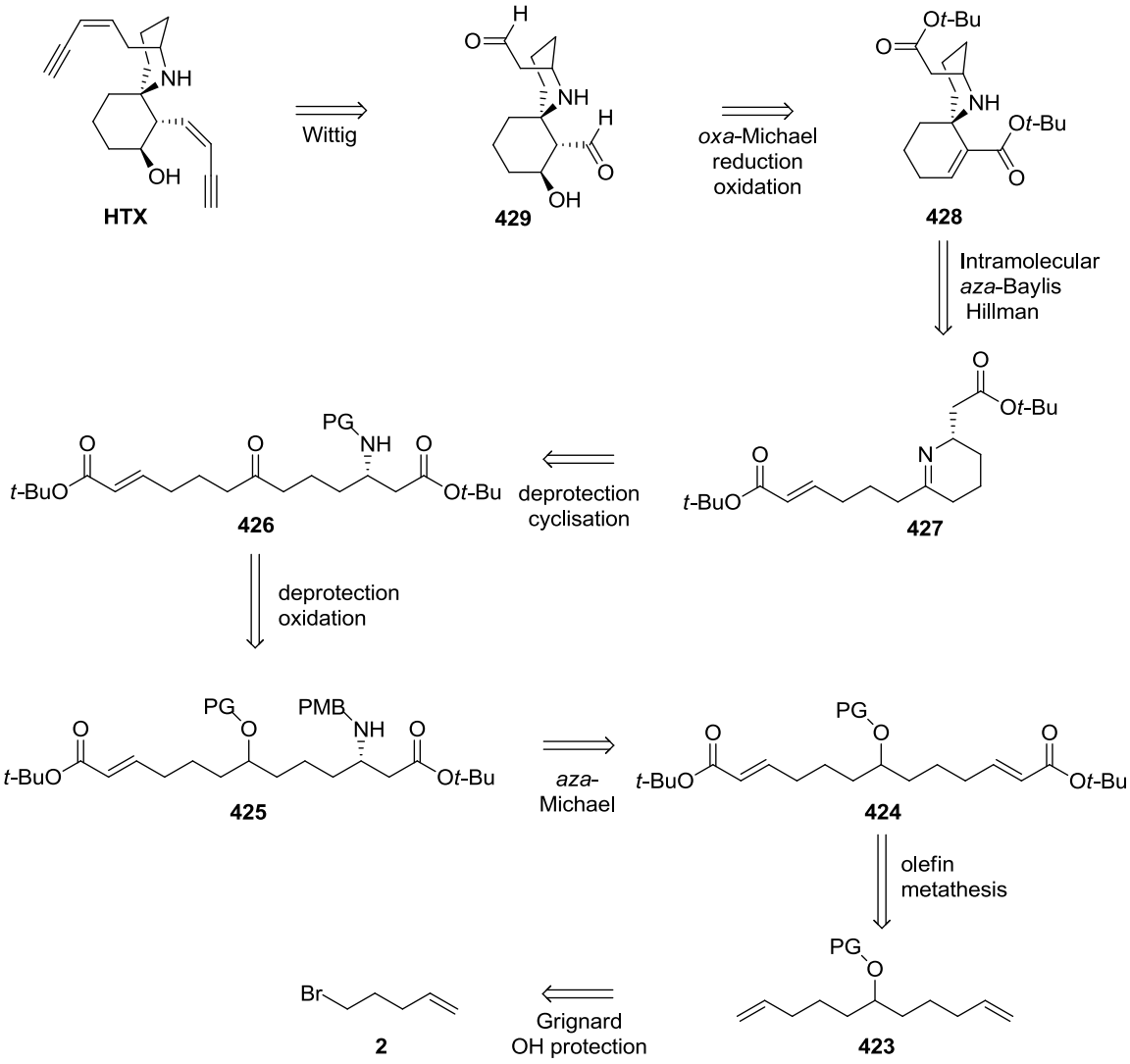
B. Stereoselective introduction of amine functionality (**424** to **425**).

C. Cyclisation and novel *aza*-Baylis Hillman spirocyclisation (**425** to **428**).

D. Stereoselective introduction of the alcohol functionality (**428** to **429**).

E. Elaboration of the *cis*-enyne side-chains (**429** to **HTX**).

Scheme 116 *Retrosynthetic analysis of* ***HTX***

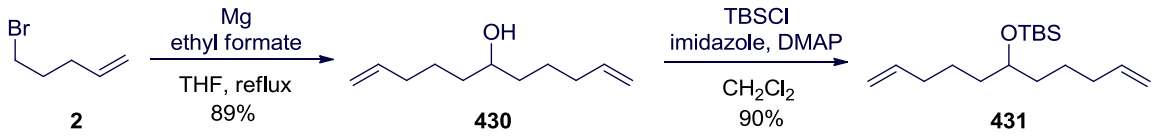


5.5 Synthesis of Acyclic Carbon Backbone

Synthesis of the **HTX** carbon backbone required high yielding robust methodology since these early steps would have to be carried out reliably on a multigram scale. If the methodology was either low yielding or insufficiently reliable, the total synthesis of **HTX** would be doomed to failure as later steps would require considerable amounts of material to trial various strategies and optimise of reaction conditions.

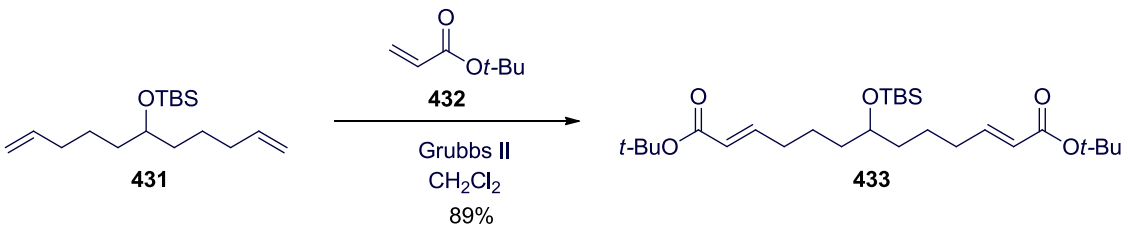
At the outset, a Grignard reaction of 5-bromo-pent-1-ene **2** with ethyl formate **3** was used to prepare alcohol **430** in 89% yield requiring no further purification (Scheme 117).[36] It was then necessary to protect the alcohol group before further functionalisation of the molecule. The TBS protecting group was chosen as it was known to be stable to Grubb's olefin metathesis and strongly basic conditions, but could be selectively removed by fluoride reagents.[37] Accordingly alcohol **430** was reacted with TBSCl under standard conditions to give protected diene **431** in 90% yield and purity after silica gel chromatography (Scheme 117).[38]

Scheme 117 *Grignard reaction and silyl protection for the synthesis of intermediate* ***431***



Diene **431** was then coupled with *tert*-butyl acrylate using Grubbs' 2nd generation catalyst, which has previously been employed to effectively couple terminal alkenes to make α,β-unsaturated esters.[39] Grubbs has observed that coupling acrylates with terminal alkenes proceeded with particularly good product selectivity.[40] He attributed this to the marked difference in reactivity between the "Type I" terminal alkene and less reactive "Type II" acrylate donors. Initially the reactive Type I alkene **431** homodimerises, which releases ethylene that escapes as a gas from the system. The Type II acrylate **432** homodimerises very slowly and before this occurs it undergoes cross-metathesis with the more reactive Type I dimer giving product **433** that is stable under the reaction conditions. It was found that after stirring the two reactants for 24 hours under ambient conditions, the desired hetero coupled product was obtained in 89% yield, only requiring a silica gel plug to remove the ruthenium impurities (Scheme 118).

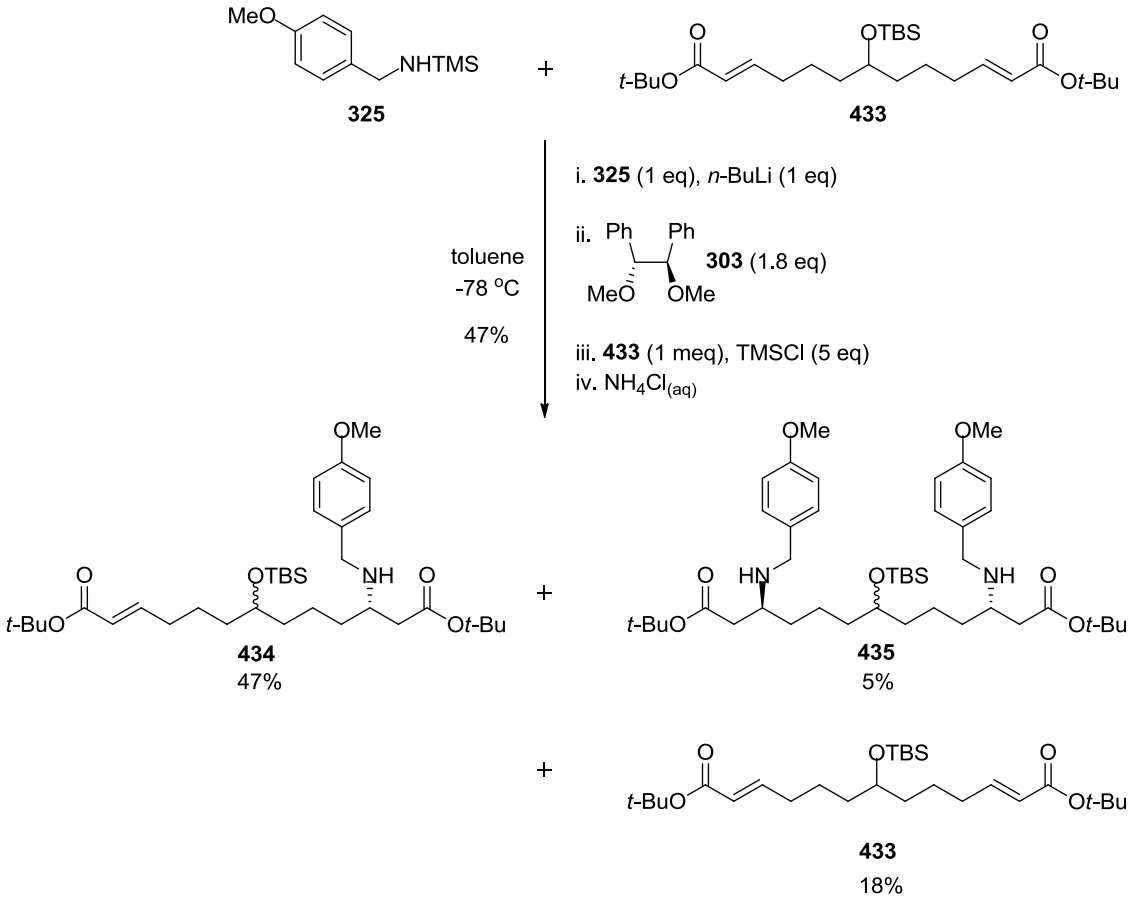
Scheme 118 *Grubbs olefin metathesis for carbon chain elongation to* ***433***



Bis-α,β-unsaturated *tert*-butyl ester **433** had therefore been synthesised in just three steps in an excellent overall 71% yield. In the proposed synthesis this thirteen carbon backbone would be used to construct the spiropiperidine ring system containing two side-chains that are suitable for homologation. To realise this synthetic approach, a nitrogen atom had to be introduced stereoselectively into the β-position to just one of the esters.

This work was achieved using the *aza*-Michael methodology described in the previous chapter, PMB amine **325** being added stereselectively and in a satisfactory yield of 47% after silica gel chromatography. The remainder of the yield was accounted for by *bis-aza*-Michael addition **435** product (5%) and unreacted **433** (18%), which could be recovered during the purification of **434** and then recycled.

Scheme 119 *Asymmetric aza-Michael reaction to introduce amine functionality*

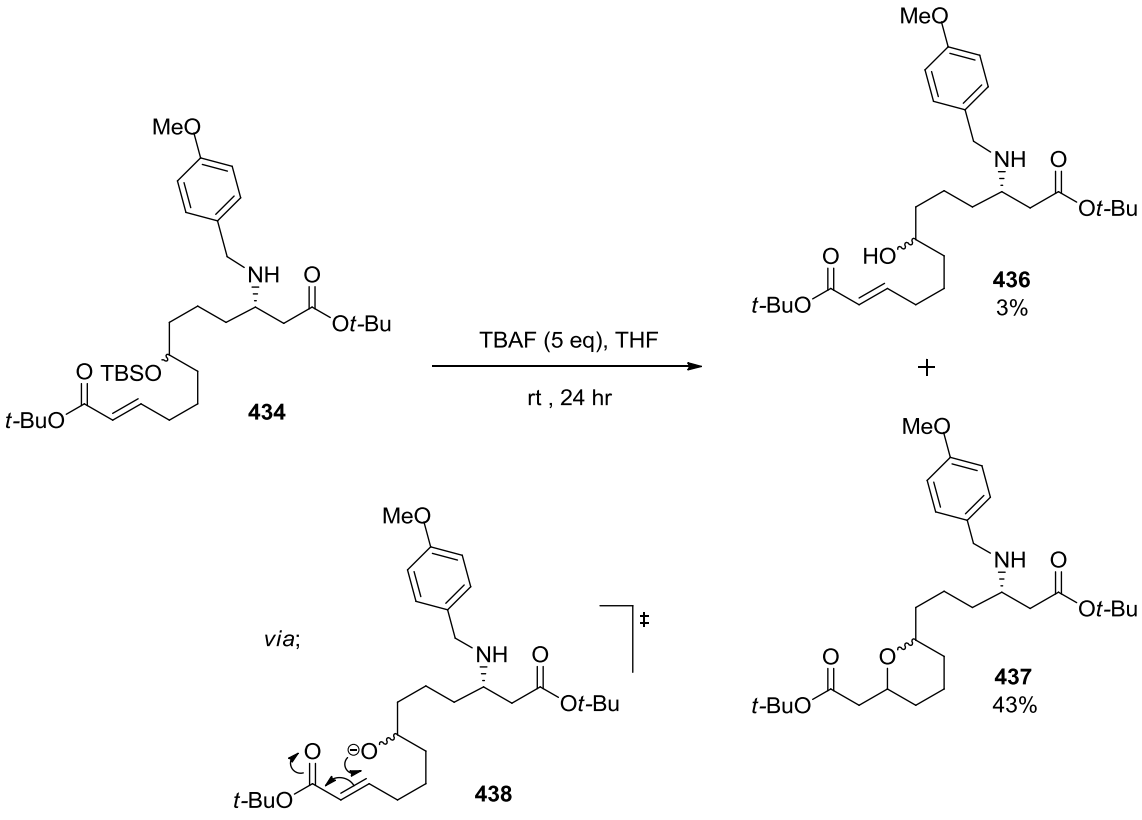


5.6 Alcohol Deprotection

Initially removal of the silyl protecting group was attempted using tetrabutylammonium fluoride (TBAF), which is often used as a water-soluble fluoride ion source for removal of silyl ether protecting groups (Scheme 120). However, the desired product **436** was isolated in just 3% yield after flash chromatography. Instead a much larger quantity of

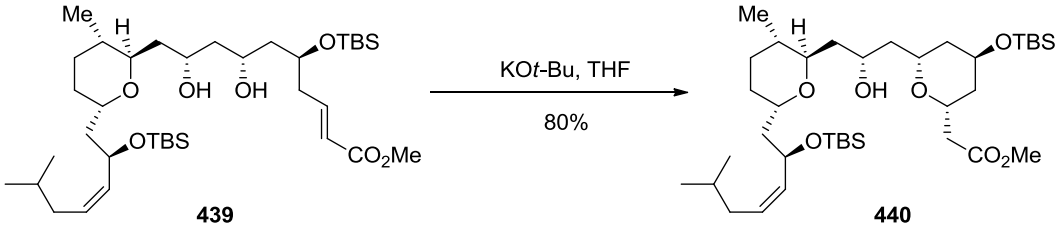
disubstituted pyran **437** was isolated (43% yield). Formation of side-product **437** was rationalised *via* intramolecular conjugate addition of the deprotected oxygen anion of intermediate **438** onto its α,β-unsaturated ester group.

Scheme 120 *Attempted TBAF deprotection of alcohol moiety of* ***434***



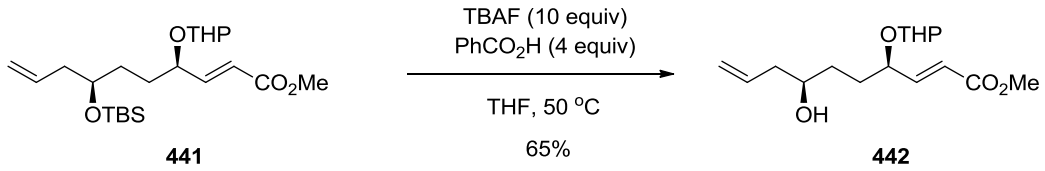
A recent review of the *oxa*-Michael reaction by Bräse and Nising highlighted that although the reaction is often reversible and suffers from low reactivity, a number of examples have been published in the literature, using various nucleophiles, where the reaction has been successfully used for the synthesis of complex molecules.[41] Indeed, examples such as the synthesis of *bis*-pyran **440** by Palmisano and co-workers (Scheme 121),[42] prompted the authors to state that this type of *oxa*-Michael reaction could be a "key tool" for the synthesis of tetrahydropyran ring systems.

Scheme 121 *oxa-Michael reaction used in the synthesis of pyran natural product*



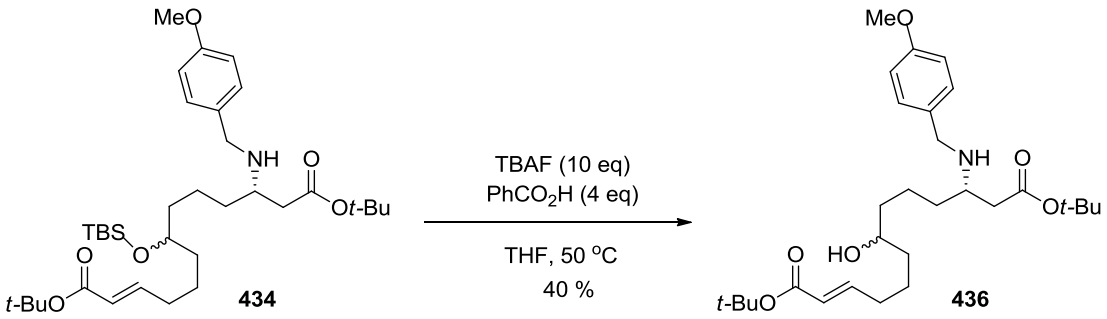
Whilst the cyclisation of anion intermediate **439** to tetrahydropyran **440** was a neat example of the intramolecular *oxa*-Michael reaction leading to a complex heterocyclic molecule, it was of no use for the synthesis of **HTX**, so alternative conditions for the silyl deprotection were sought. A careful literature search revealed that the Kibayashi group had encountered a similar problem during their synthesis of the natural product (-)-Vermiculine (Scheme 122).[43] When desilylation of **441** was carried out with TBAF in THF at 60 °C they also isolated the *oxa*-Michael product. However, by using a benzoic acid buffered THF solution of TBAF, the de-protected product **442** could be obtained in 65% yield.

Scheme 122 *Kibayashi buffered TBAF deprotection of* ***441***



Kibiyashi's method was then successfully applied to the deprotection of **434** (Scheme 123). The yield of alcohol **436** after purification by silica gel flash chromatography was low at 40%, but subsequent trials proved it to be a reliable protocol.

Scheme 123 *Successful buffered TBS deprotection of* ***434***

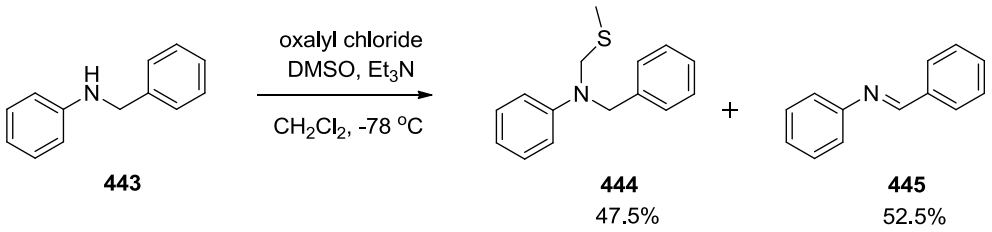


5.7 Oxidation attempts

A great range of reagents and conditions are available to the organic chemist for the oxidation of secondary alcohols such as **436** to ketones: chromium reagents (Jones's reagent,[44] Collins's reagent,[45] Corey's reagent[46-47]); potassium permanganate;[48] tetra-

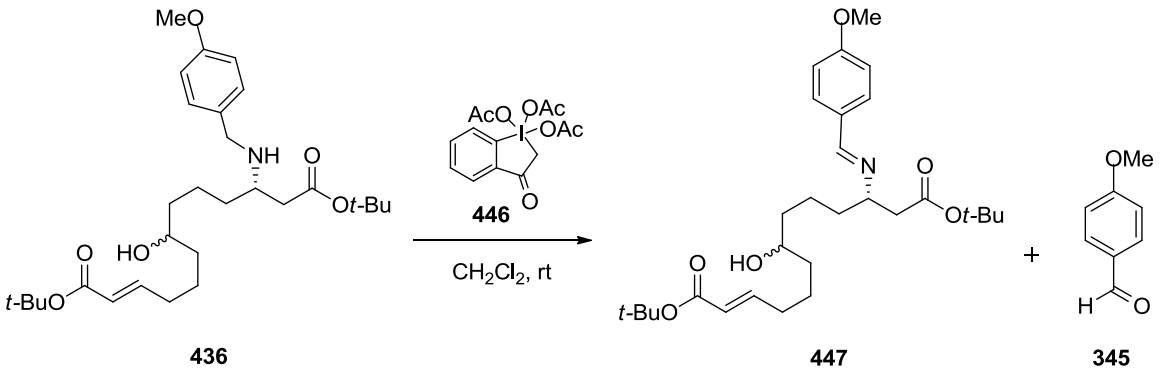
propylammonium perruthenate (TPAP);[49] hypervalent iodine reagents;[50] manganese dioxide;[51] and metal transfer hydrogenation catalysts[52-53] to name but a few. Initially the Swern oxidation conditions were trialed as a mild oxidant for the oxidation of secondary alcohol **436**. Disappointingly, the standard methodology gave a complex mixture of products after complete consumption of the starting material. Silica gel chromatography failed to isolate any of the desired product, but the isolation of *para*-methoxybenzaldehyde **345** suggested that the oxidative conditions had resulted in oxidation of the PMB protected amine functionality. Although not anticipated, this additional oxidation is not wholly surprising since the oxidative deprotection of PMB amines by oxidants such as CAN and DDQ is well-known in the literature.[54] In fact, Overton and co-workers have previously shown that standard Swern oxidation conditions convert phenyl benzylamine **443** to methylthiomethyl amine **444** and imine **445** (Scheme 124).[55]

Scheme 124 *Swern oxidation of phenyl benzylamine **443***



The oxidation of alcohol **436** was next trialed with the Dess-Martin periodinane reagent **446** (DMP), since DMP has been found to be a mild oxidant that shows good chemoselectivity.[56] 1.1 equivalents of DMP were used for oxidation of alcohol **436** for one hour, before quenching and extraction gave a crude product that was a mixture of starting material **436**, imine **447** and benzaldehyde **345** in a ratio of 1:1.75:1 (Scheme 125).

Scheme 125 *Attempted oxidation of secondary alcohol **436***

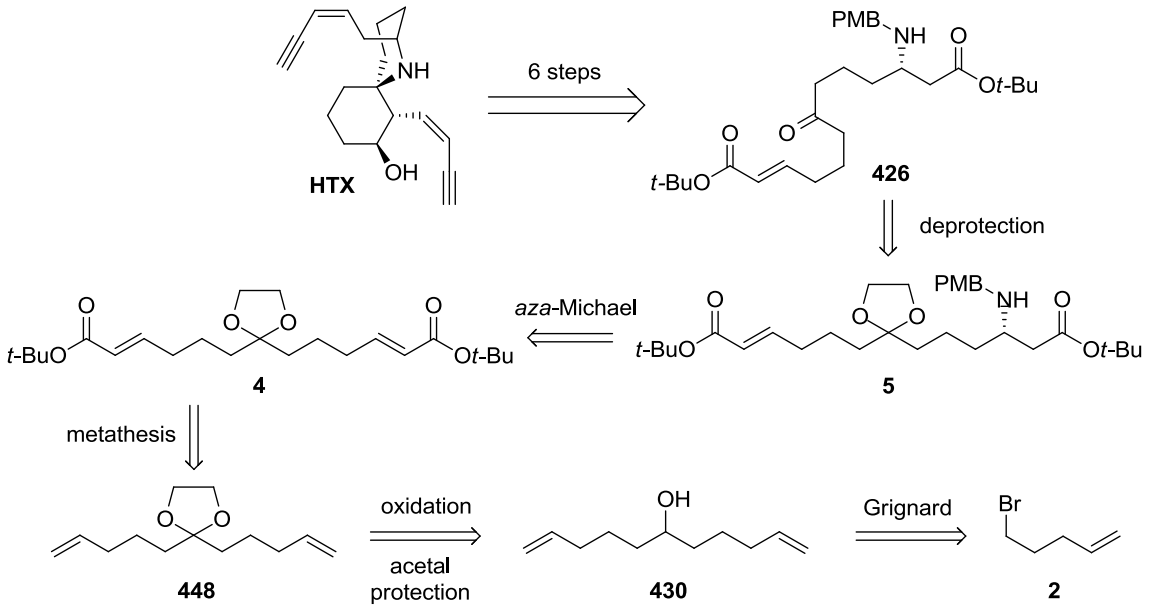


2-Iodoxybenzoic acid (IBX) was then trialed as an oxidant of alcohol **436** since this alternative hypervalent iodine reagent has been reported to be even milder than DMP for some reactions (e.g. the cleavage of 1,2-diols).[57] After stirring the reaction mixture, consisting of a DMSO solution of starting material and 1.4 molecular equivalents of IBX, for 1 hour only unreacted starting material was recovered. The reaction was then put back on with excess IBX (five equivalents) and heated incrementally whilst following the reaction progress by TLC. Despite heating the reaction at 150 °C for six hours, the starting material **436** remained unreacted, with oxidant degradation preventing any further reaction.

5.8 Alternative Protecting Group Strategy

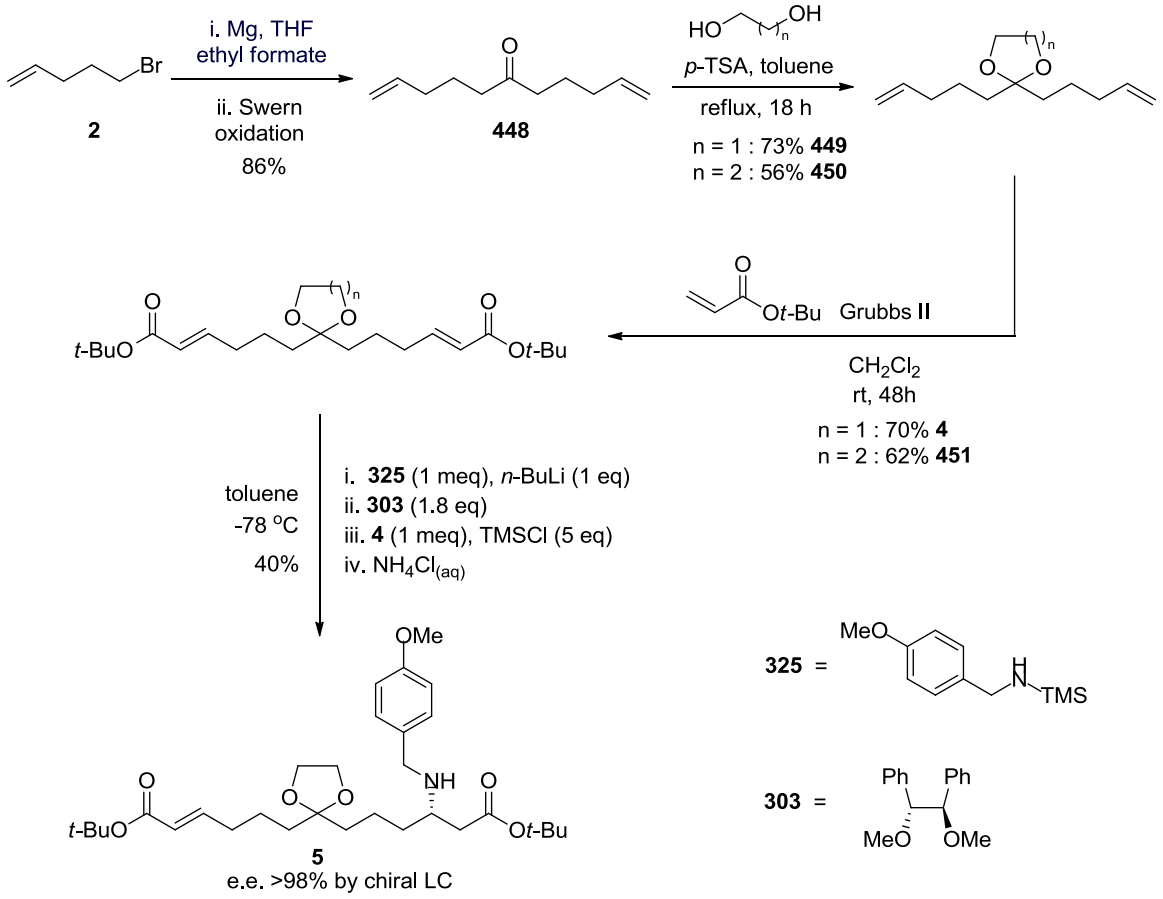
After these oxidation trials, the reserves of alcohol **436** had run out and it seemed an opportune time to review the synthetic strategy. After just five synthetic steps, the thirteen carbon backbone had been synthesised and the amine functionality had been introduced stereoselectively. Synthetic efforts could now be focused on the re-synthesis of advanced alcohol intermediate **436** and screening for a chemoselective oxidation methodology. Alternatively, the synthetic strategy could be modified to overcome the lack of chemoselectivity encountered in the alcohol oxidation step in the presence of the PMB protected amine functionality. The latter option was favoured and a strategy was designed that incorporated earlier alcohol oxidation followed by protection of the ketone functional group as an acetonide, followed by olefin-metathesis and *aza*-Michael methodologies for rapid carbon chain amplification and desymmetrisation (Scheme 126).

Scheme 126 *Alternative retrosynthetic strategy of* ***HTX***



Accordingly alcohol **430** was re-synthesised using Grignard methodology as described previously, and then oxidised under Swern oxidation conditions, which furnished ketone **448** in 97% yield. Both the 1,3-dioxane **449** (73%) and 1,3-dioxalane **450** (56%) acetonides were synthesised *via* treatment with the corresponding diol in the presence of *p*-TSA in toluene. Acetonides **449** and **450** were coupled with *tert*-butyl acrylate **432** using Grubb's 2nd generation ruthenium catalyst in CH_2Cl_2 to afford bis-α,β-unsaturated esters **4** (70%) and **451** (62%). The synthesis of 1,3 dioxane acetal **449** and subsequent alkene metathesis proceeded in higher yield, so considering this protecting group is also more labile to acid promoted deprotection, it became the acetal protecting group of choice. Therefore **4** was then reacted with amine **325** using the previously described chiral ligand mediated **303** *aza*-Michael methodology to afford amine **5** in 40% yield and >97% e.e. (Scheme 127).

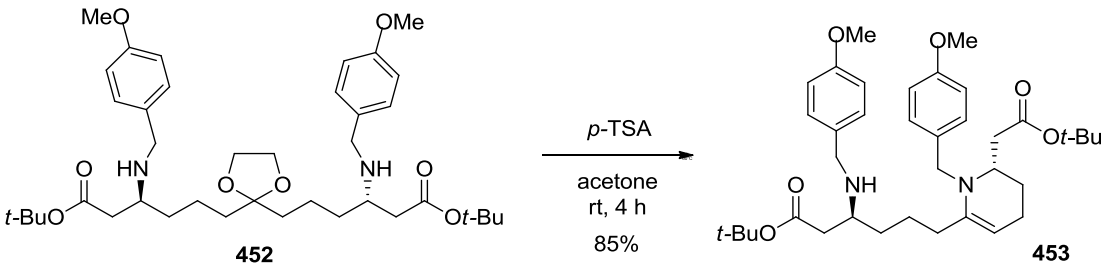
Scheme 127 *Acetal protection and elaboration*



5.9 Investigation into Acetal Deprotection

Two initial attempts at acetal deprotection of *aza*-Michael product **5**, using AcOH/water and *p*-TSA/acetone conditions respectively, yielded complicated poorly resolved ^{1}H NMR spectra. These same conditions were then tested on *bis-aza*-Michael product **452** in the hope that this symmetrical system could clarify what was occurring under these acidic conditions (Scheme 128). Frustratingly, the ^{1}H NMR spectrum of the crude product was poorly resolved and very complicated. However, analysis of the crude products of these reactions by mass spectrometry, revealed disappearance of starting material **452** and emergence of a new peak with a m/z ratio of 623.4095 - a mass loss of 61.5 that suggests acetal deprotection and accompanying dehydration. This result suggested that not only had the acetal group been removed, but that the amino group had reacted intramolecularly with the resultant ketone group to afford a cyclic structure. Further analysis of the products by ^{1}H, ^{13}C and 2D experiments in $CDCl_3$ was hampered by the poor quality of the resultant spectra, however screening alternative deuterated solvents revealed that toluene-d_8 gave much cleaner ^{1}H and ^{13}C NMR spectra that could be properly analysed. This spectral data indicated that the structure of the major product was enamine **453**.

Scheme 128 *Acid catalysed acetal deprotection of bis-aza-Michael product **452***



The *p*-TSA/acetone deprotection method was repeated on *aza*-Michael product **5** and the isolated product was analysed in toluene-d_8 (Scheme 129). Now it was apparent that the acetal singlet peak had disappeared from both the ^{1}H and ^{13}C spectra, clearly showing successful acetal deprotection had occurred. The structure of the product could be assigned as cyclic enamine **454** with the aid of key diagnostic peaks. In the ^{13}C NMR spectrum resonances at 140.5 ppm and 99.6 ppm are due to the enamine carbons C(5) and C(10) (Figure 35), a conclusion that is confirmed by a broad resonance at 4.51 ppm in the ^{1}H spectrum that is characteristic of the enamine H_A. Careful analysis of cross-peaks in the COSY spectrum (Figure 36) suggested that the enamine double bond was

endocyclic, since the resonance at 2.39 ppm, which is due to H_E, interacts with a resonance centred at 1.82 ppm and 1.50 ppm. If the double bond was exocyclic H_E would interact with resonances further downfield than 1.50 ppm as the adjacent protons would be alkenyl and allylic protons. This conclusion agrees with previously reported assignments for related 6-alkyl-1,2,3,4-tetrahydropyridines that were found to be endocyclic enamines.[58-61]

Scheme 129 *p-TSA catalysed acetal deprotection of aza-Michael product* ***5***

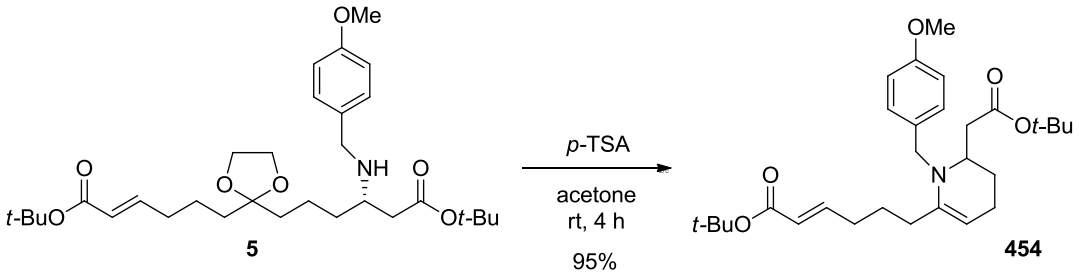


Figure 35 *Annotated* ^{13}*C NMR spectrum of cyclic enamine* ***454***

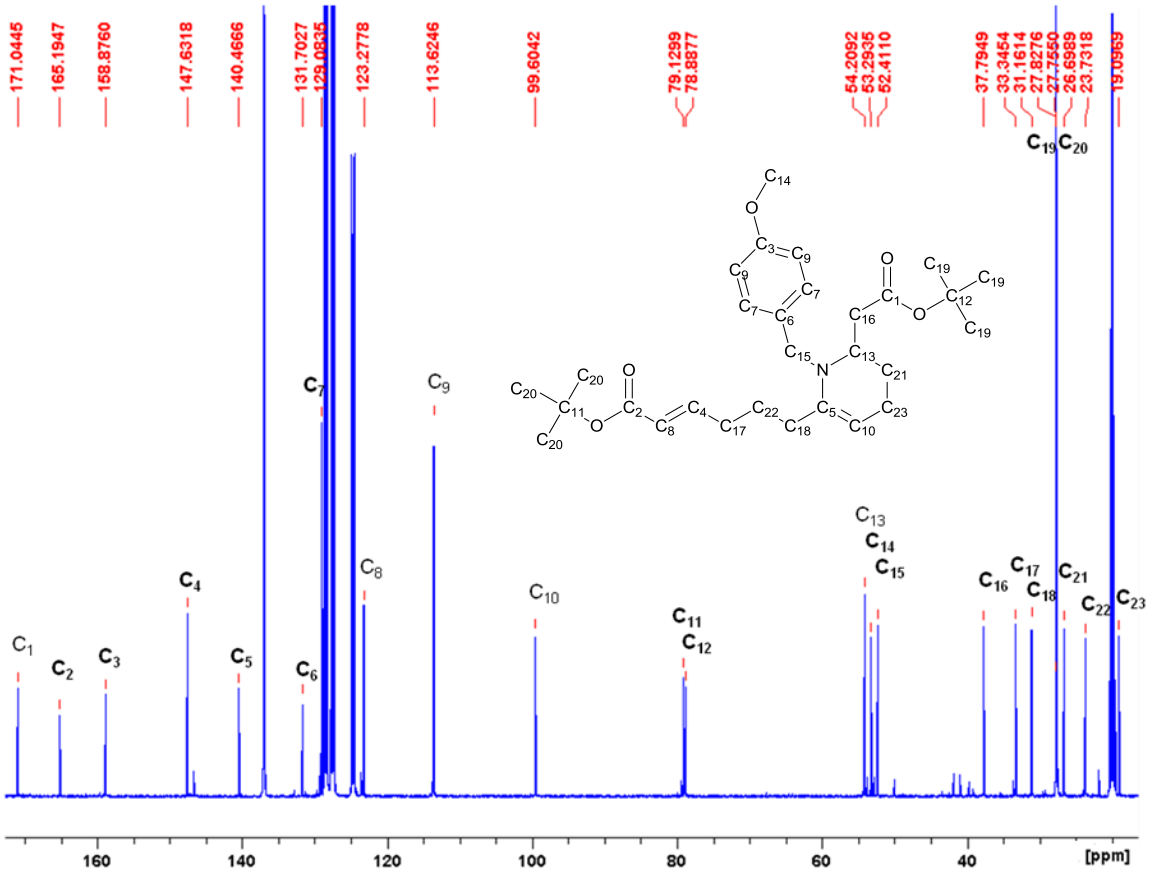
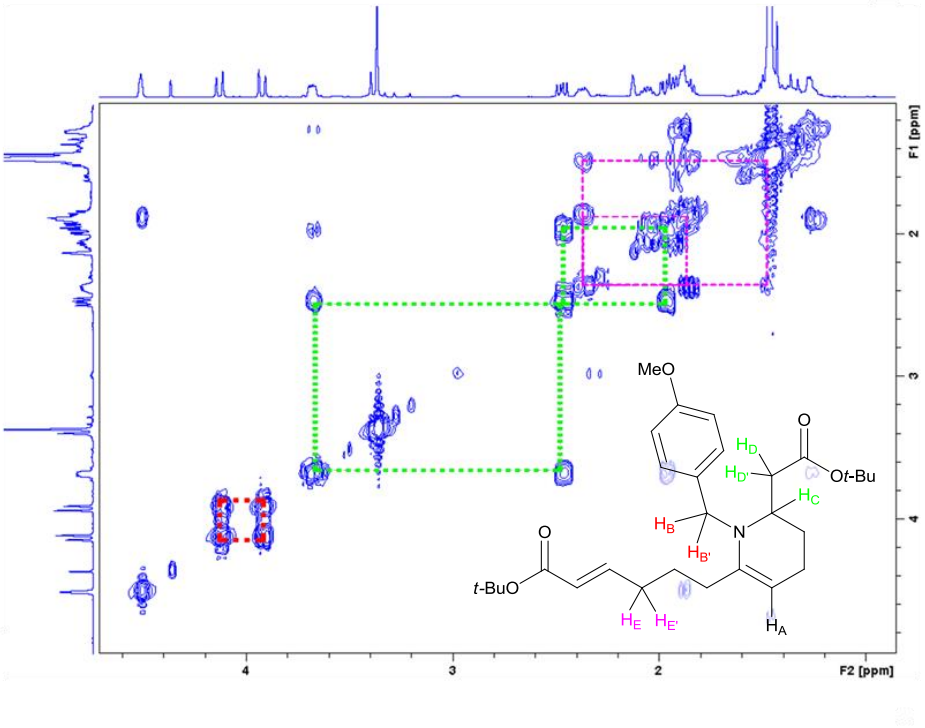


Figure 36 *COSY spectrum showing key interactions for cyclic enamine* ***454***



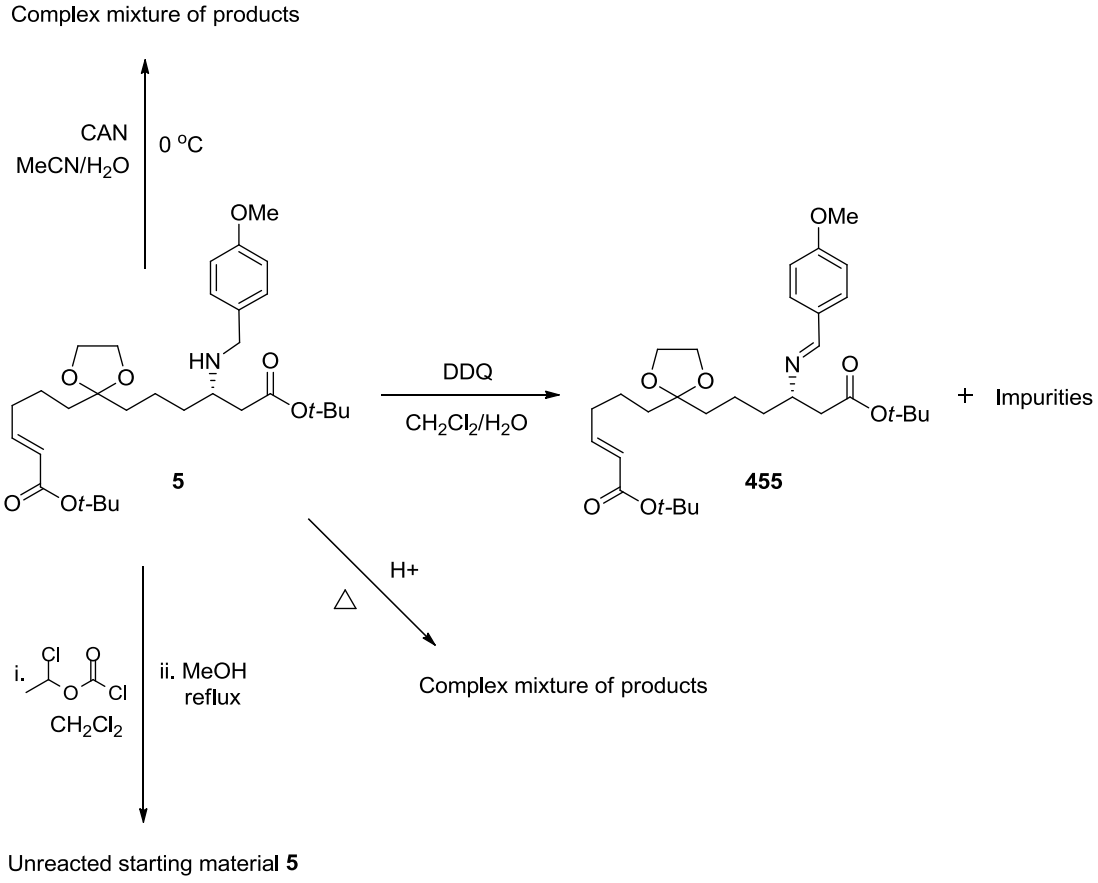
5.10 Amine Deprotection

Initially, deprotection of the PMB group of amine **5** was trialed using standard oxidative CAN methodology.[54] The reaction yielded a complex mixture of products in low yield that could not be fully characterised, although *para*-methoxybenzyaldehyde could be clearly seen in the ^{1}H NMR spectrum, showing that some PMB deprotection had occurred. However, it was also clear that the acetal resonance at 3.8 ppm was no longer present, whilst a resonance at 4.8 ppm that was characteristic of cyclic enamine formation was visible. DDQ oxidative deprotection was also trialed, but did not yield the desired product. Instead it gave mixtures of starting material **5**, benzaldehyde **345** and imine **455**. As an alternative to oxidative deprotection, some other deprotection methods were investigated. Strong acid has been used to deprotect 2,4-dimethoxybenzylamines and these conditions have been employed to deprotect PMB amines.[62] Therefore, **5** was heated in a TFA solution or in a (1.25 M) methanolic HCl solution, and in both cases acetal deprotection and ester hydrolysis occurred. However, the PMB group was not deprotected. α-Chloroethyl choroformate has been shown to deprotect the PMB group from 2^{o} amines.[63-64] This reagent was first trialed for the deprotection of **5**, with just acetal deprotection observed, and then on enamine **454**, which yielded unreacted starting material despite the use of dichloroethane in place of CH_2Cl_2 and forcing reflux conditions.

The PMB protecting group can be removed, in an analogous fashion to benzyl protected amines, under reductive conditions.[65] Unfortunately, these conditions also reduce alkene

bonds. However, it was reasoned that amine deprotection with concomitant alkene reduction would allow the advanced structure **457** to be synthesised that could then be used to trial spirocyclisation conditions. Accordingly the alkene bond of **5** was first reduced in near quantitative yield by cobalt chloride hexahydrate mediated sodium borohydride reduction,[66] and then the PMB group reduced by hydrogenolysis using one atmosphere of hydrogen and Pearlman's catalyst (Scheme 131).[65] Compound **456** was then subjected to the *p*-TSA acetal deprotection and cyclisation methodology developed previously for **452**, which to our delight gave cyclic imine **457** in good yield. The ^{1}H NMR spectrum of **457** shows that the aromatic and acetal resonances are absent and CH_A resonance has shifted downfield to 3.83 ppm (Figure 37). It is clear that **457** exists solely as an imine in toluene-d_8, since there is no broad enamine resonance centred around 4.5 ppm in the ^{1}H NMR spectrum (as seen for compounds **453** and **454**) and the ^{13}C NMR spectrum has no enamine peaks (Figure 38), but instead imine carbon C(3) resonance at 168.1 ppm adjacent to the carbonyl carbon resonances of C(1) and C(2) at 172.0 ppm and 170.8 ppm.

Scheme 130 *PMB amine deprotection and cyclisation*



Scheme 131 *Reductive deprotection of PMB group of amino-acetal* ***5***

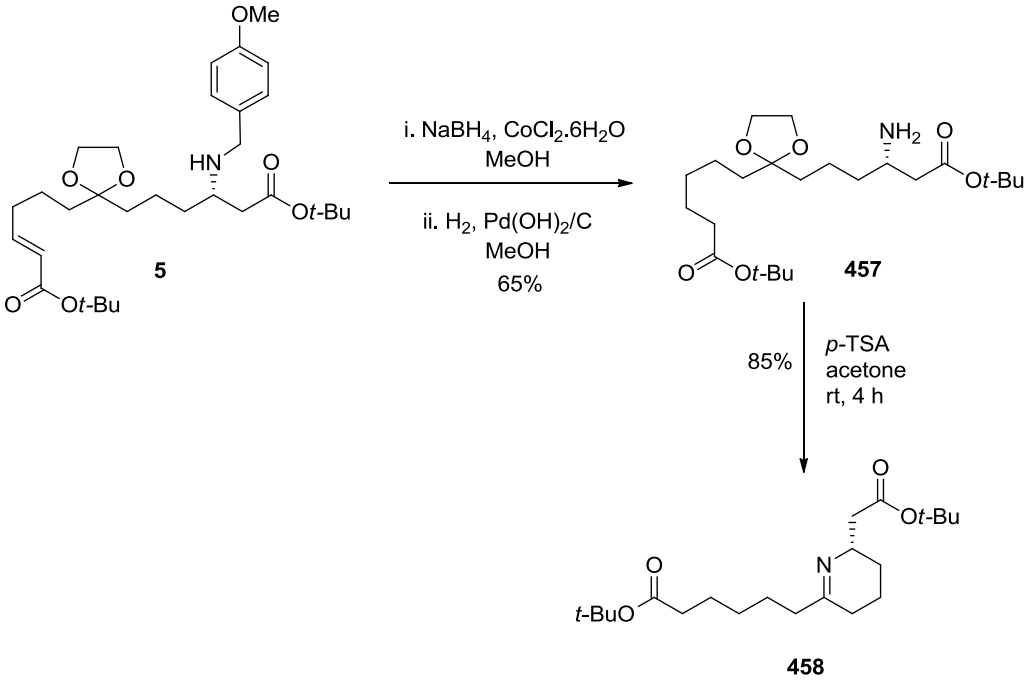


Figure 37 *Annotated* ^{1}H *NMR spectrum of cyclic imine* ***458***

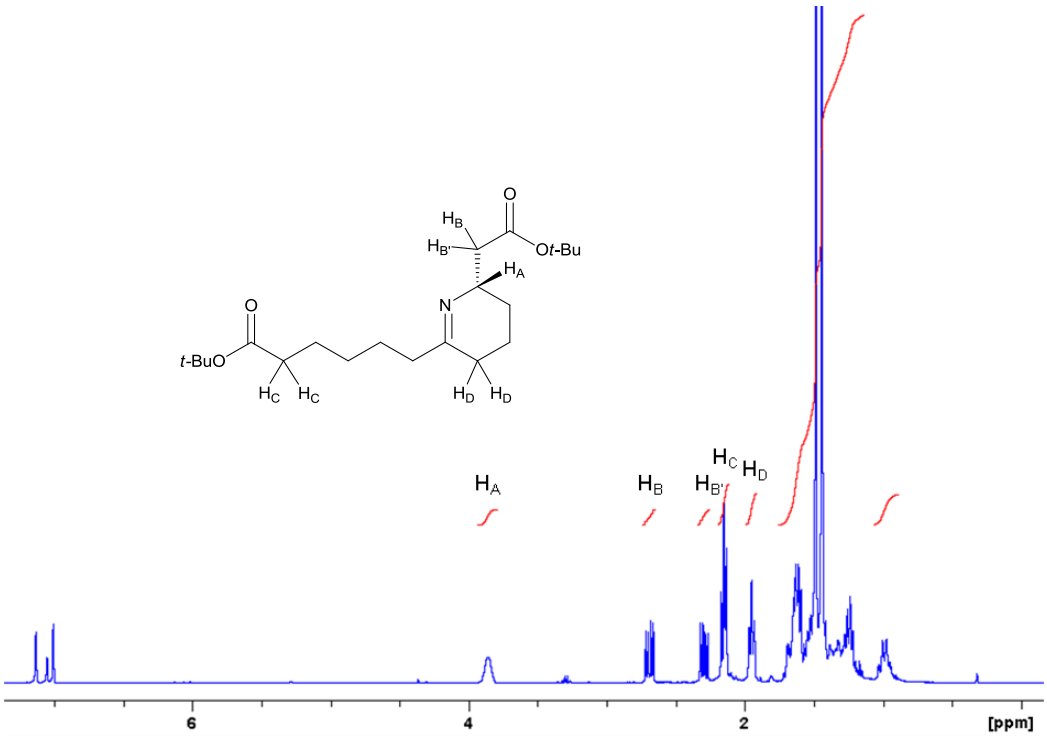
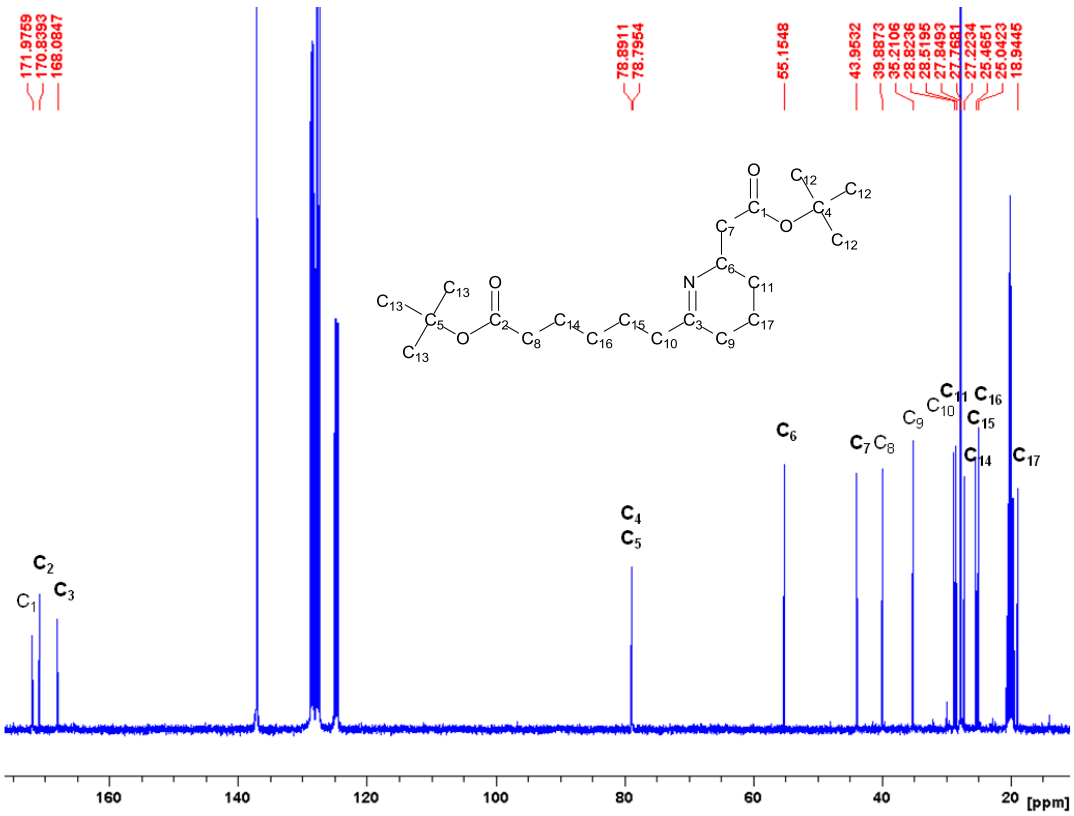


Figure 38 *Annotated ^{13}C NMR spectrum of cyclic imine* ***458***

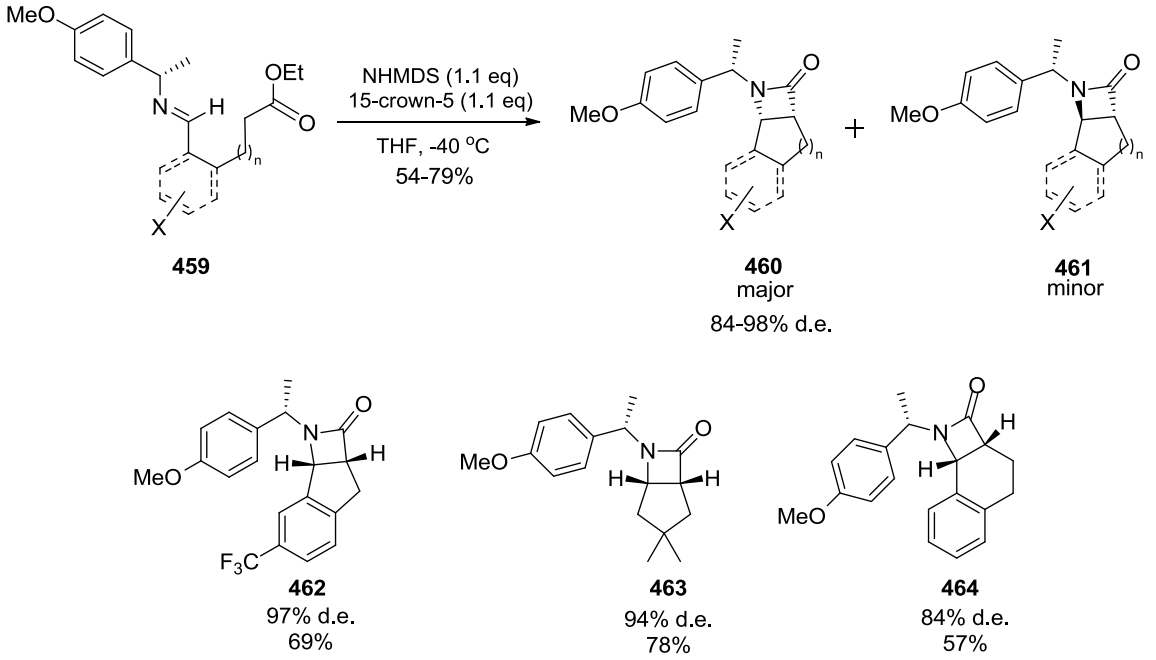


5.11 Attempted Spirocyclisation *via* Ester Enolate-Imine Reactions

Recently, the Bull group has reported the first diastereoselective intramolecular ester-enolate imine cyclisation reaction to afford cyclic β-lactams in high d.e. (Scheme 132).[2] This cyclisation reaction was shown to be highly diastereoselective for cyclisation of a range of: (i) substituted aryl ω-imino esters (n = 1), giving 90-97% d.e.; (ii) aliphatic ω-imino esters (n = 1), giving their corresponding β-lactams in 92-94% d.e.; and for an aryl ω-imino ester containing an increased chain length (n = 2), affording **464** in a reduced yield (57%) and diastereoselectivity (84% d.e.).

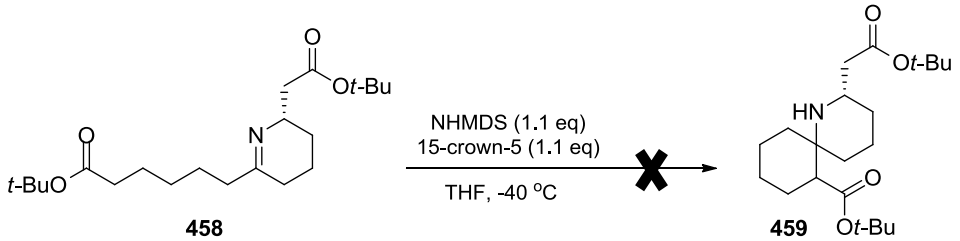
In order to achieve spirocyclisation, this base promoted ester-enolate imine cyclisation methodology was investigated using imine **458** as the substrate (Scheme 133). Initially **458** was subjected to the optimised ester-enolate imine cyclisation conditions.[67] Therefore, a THF solution of **458** was treated with NHMDS (1.1 meq) and 15-crown-5 (1.1 meq) and stirred at -40 °C for 24 hours. Then a standard work-up procedure was followed and yielded unreacted starting material **458**. Unperturbed by this negative result, the reaction was repeated at room temperature and then at 40 °C, but again only unreacted

Scheme 132 *Intramolecular stereoselective ester-enolate imine cyclisation reaction, depicting representative products* ***462-464***



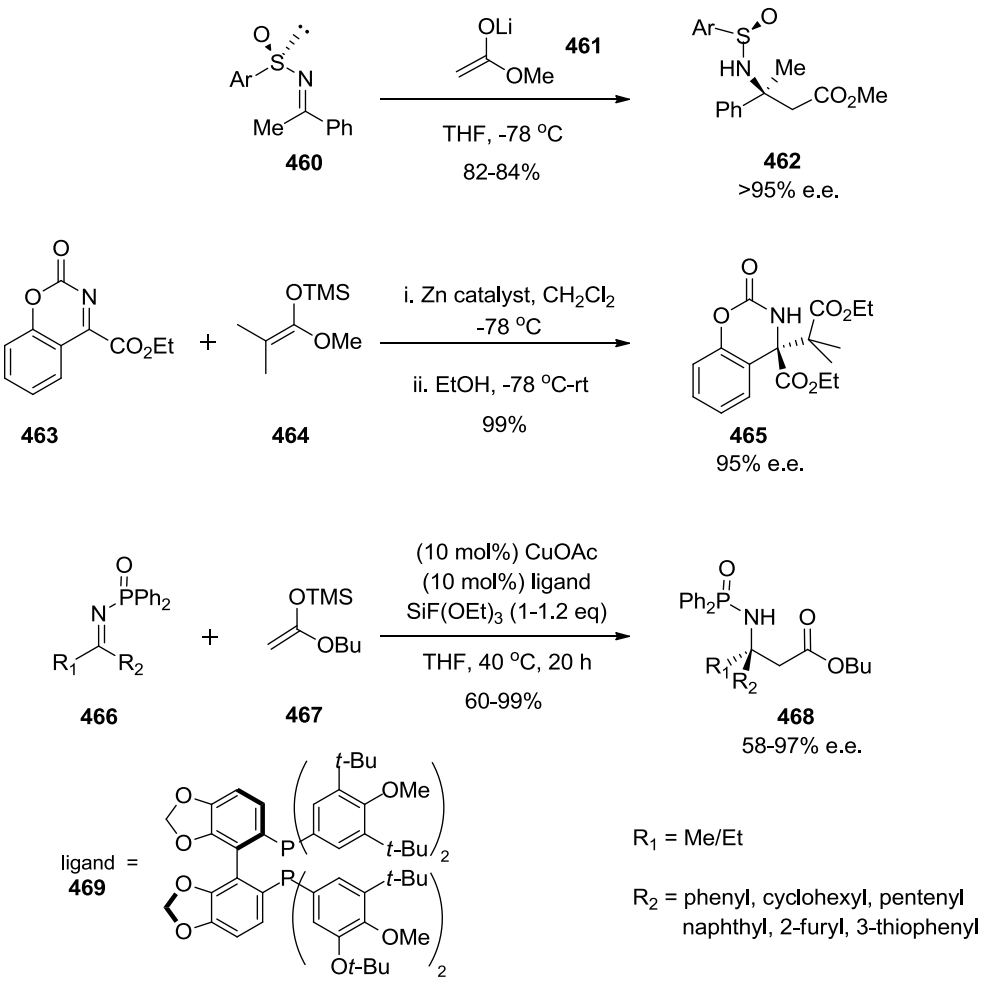
starting material was recovered. The reaction at room temperature was repeated and quenched with D_2O after one hour to ensure that deprotonation was occurring. In-process LC/MS analysis and the ^1H NMR spectrum of the crude product clearly showed that inclusion of deuterium was occurring. The reaction was then repeated at room temperature using fresh NHMDS without the crown ether additive without any change in the result. The influence of the base was further investigated by using LHMDS and LDA in place of NHMDS, and then by increasing the equivalents of NHMDS to 2.2. In all cases only unreacted starting material **458** was recovered.

Scheme 133 *Base promoted ester-enolate imine spirocyclisation trials*



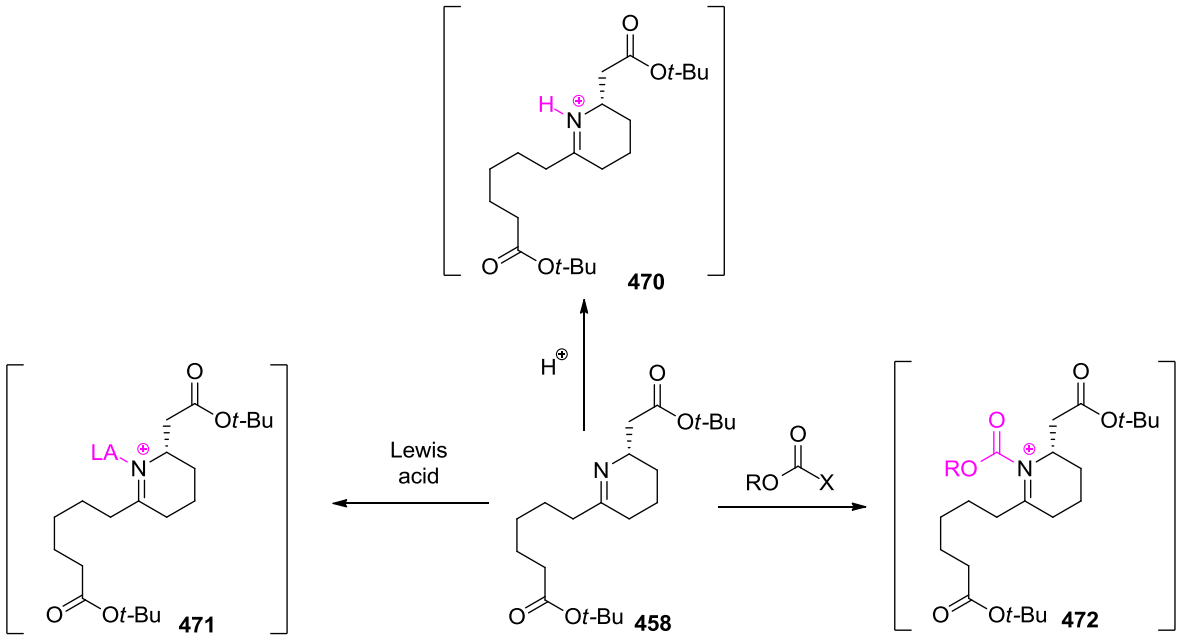
A careful search of the literature showed that although the first example of the asymmetric intramolecular ester-enolate imine condensation had only recently been reported by Bull and co-workers, there were many reports and comprehensive reviews that describe the intermolecular version of this type of enolate-imine cyclisation reaction.[68-69] A review of these reports revealed that almost all of these reports descibed the reaction of ester-enolates with aldimines, with only three examples reported of the reaction of ester-enolates with ketimines (Scheme 134),[70-72] and only a few examples of ketimines reacting with other nucleophiles.[73-78] Researchers have highlighted two major difficulties for the reaction of nucleophiles with ketimines: the steric hindrance around the ketimine bond that makes new bond formation to a ketimine sp^2-carbon unfavourable; and the fact that the ketimine group has acidic alpha protons that are rapidly isomerized under basic conditions to afford an unreactive enamine anion. In each of the three examples shown in Scheme 134 an electron withdrawing group was required to be bound to the ketimine nitrogen for the ketimine bond to undergo nucleophilic attack.

Scheme 134 *Literature examples describing addition of ester-enolates to ketimines*



It was reasoned that the main reason for the lack of cyclisation of the enolate of imine **458** was due to the relatively low reactivity of the ketimine bond. Therefore, three activation strategies were considered for the potential activation of the ketimine bond nucleophilic attack (Scheme 135): Brønsted acid catalysis; Lewis acid catalysis; and derivatisation of imine **458** to afford an *N*-acyliminium ion.

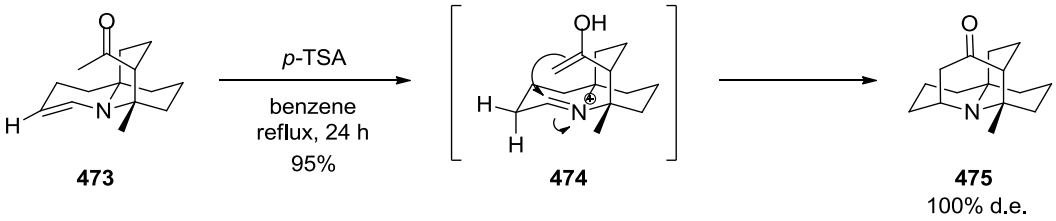
Scheme 135 *Potential ketimine activation strategies*



5.12 Attempted Brønsted Acid Catalysed Spirocyclisation

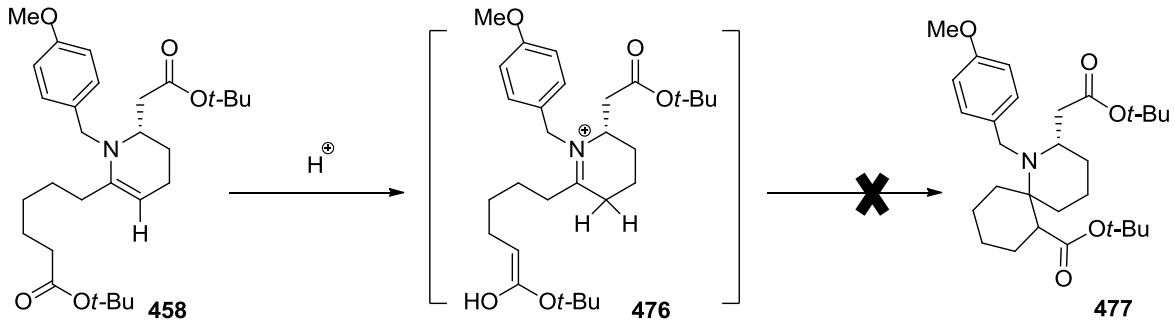
Canet and co-workers have shown that *p*-TSA could be used as a Brønsted acid to catalyse the Mannich reaction of ketones to aldimines for the formation of 2,6-disubstituted piperidines (Scheme 136).[79-80] This Brønsted acid activation has subsequently been used by a number of other groups for the Mannich reactions of ketimines for the synthesis of ring fused polycyclic alkaloids,[81-84] and the Corey group have used this activation strategy for a spirocyclisation route to an unnatural HTX analogue (Section 5.3.3).[20] These reports use a variety of acids, solvents and conditions to achieve intramolecular cyclisation by nucleophilic attack of an enol species at a charged iminium ion centre. Interestingly many of these intramolecular cyclisation reactions are highly stereoselective due to their transition states adopting well-defined conformations that exhibit a pronounced influence on the stereochemical outcome.

Scheme 136 *Example of intramolecular Brønsted acid catalysed Mannich-type reaction for the synthesis of tetracyclic fused-ring natural product rac-Porantherine* ***475***[82]



Therefore, the spirocyclisation of **458** was attempted using a series of Brønsted acid conditions. Four Brønsted acids in various solvents at room temperature, 50 °C and 100 °C were screened for their ability to catalyse the spirocyclisation of enamine **458** (Table 14). Treatment of **458** with (1.25 M) methanolic HCl led to partial transesterification to the corresponding methyl esters at room temperature and full transesterification at 50 °C (Entry 1). H_2SO_4 in MeCN (or DMSO) resulted in hydrolysis of the ester groups of **458**, with no evidence of any spirocyclisation having occurred (Entry 2 and 3). Treating with TFA in DMSO gave no reaction (Entry 4), whilst TFA in toluene gave a intractable sticky brown solid as did *p*-TSA in toluene (Entry 5 and 6).

Table 14 *Attempted Brønsted acid catalysed spirocyclisation of enamine* ***458***



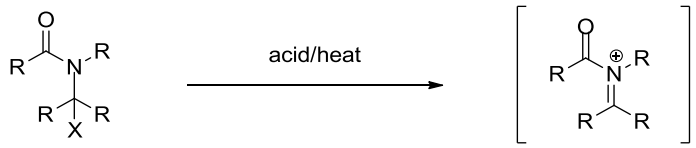
Entry	Acid	Solvent	Result
1	[a]HCl	Methanol	Partial-complete transesterification
2	[b]H_2SO_4	MeCN	Ester hydrolysis
3	[b]H_2SO_4	Dioxane	Ester hydrolysis
4	[c]TFA	DMSO	No reaction
5	[c]TFA	Toluene	Insoluble brown solid
6	[d]*p*-TSA	Toluene	Insoluble brown solid

Reactions run on a 0.02 mmol scale at rt, 50 °C and 100 °C for 24 hours under N_2. (a) 1.25M solution; (b) 0.05M solution; (c) 30 eq.; (d) 1 eq.

5.13 Attempted Spirocyclisation of *N*-Acyliminium Ions

N-acyliminium ions **479** have been shown to be versatile reactive electrophiles and have been the subject of a number of reviews in recent years.[89-93] Because of their high reactivity they are nearly always generated *in situ* from more stable precursors of the general structure **478**, with acid or heat employed to break a C-X bond (Scheme 137). When X is an alkoxy substituent, the precursor is generally a stable molecule requiring the addition of a Brønsted or Lewis acid to break the C-*O* bond and generate the *N*-acyliminium ion (Scheme 138 and 139).

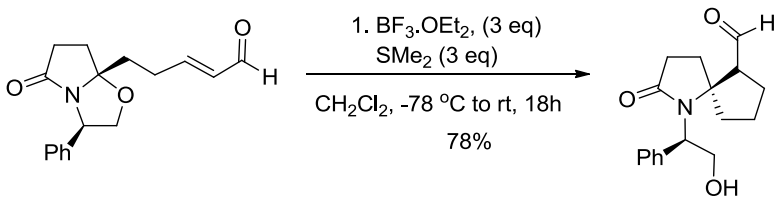
Scheme 137 *Standard in situ generation of N-acyliminium ion*



478 **479**

For example, Aggarwal and co-workers have shown that *N*,*O*-aminal amide **481** can be used to generate an *N*-acyliminium ion by treatment with $BF_3.OEt_2$, which then undergoes a Baylis-Hillman reaction mediated by dimethyl sulfide to afford a [4.4] spirocycle (Scheme 138).[94] This suggests that if a similar *N*,*O*-aminal amide of imine **458** could be synthesised then it might undergo an intramolecular Mannich-type reaction to from a spirocycle, and also that this methodology could potentially be applied to the Baylis-Hillman reaction required for the synthesis of **HTX**.

Scheme 138 *Intramolecular Baylis-Hillman reaction of N-acyliminium ion generated in situ from N,O-aminal amide **488***

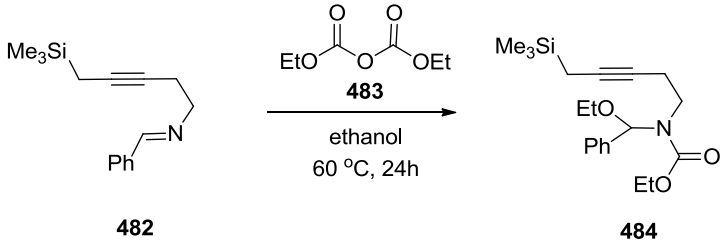


480 **481**
82:18

N,*O*-acylaminals can be synthesised in a number of ways: by the intramolecular reaction of amides with aldehydes to generate a five- or six-membered ring;[95] the partial reduction of imides by DIBAL or $NaBH_4$;[96] the addition of one equivalent of a Grignard reagent to an

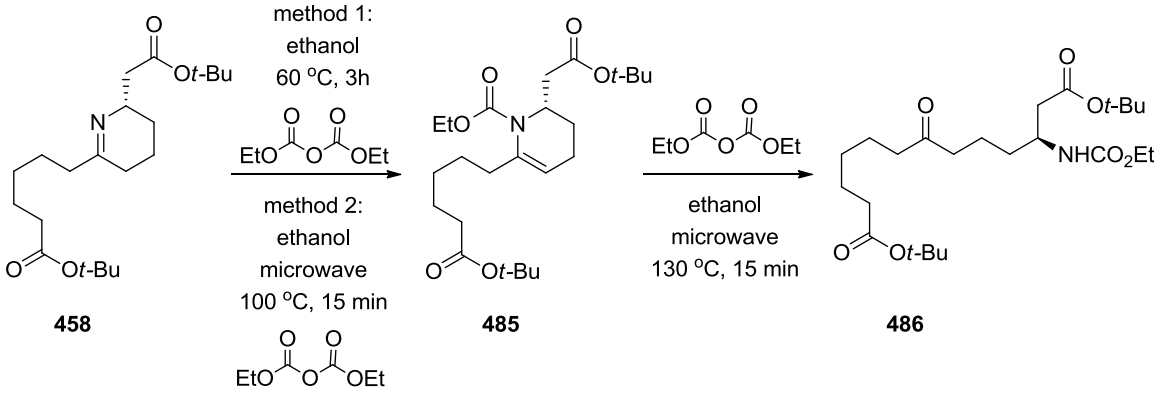
imide;[97] or the electrochemical oxidation of amides, carbamates and N-acylated amino acids.[98-99] Unfortunately the synthesis of 4° *N*,*O*-acylaminals, much like the reaction of nucleophiles with ketimines rather than aldimines, is not so trivial due to both the increased steric strain and the presence of enolisable acidic alpha protons. In fact there are only a handful of examples where the synthesis of 4° *N*,*O*-acylaminals has been reported.[100-106] Of these the synthesis of *N*,*O*-aminal **484** by the treatment of imines with diethyl pyrocarbonate **483** reported by Speckamp appeared to be most relevant (Scheme 139).[107]

Scheme 139 *Speckamp synthesis of N,O-acylaminal **492***



When this methodology was applied to imine **458**, the desired *N*,*O*-acylaminal was not isolated. Instead enolisation of intermediate *N*-acyliminium ion occurred before the intermediate iminium species could be trapped by an ethoxide anion, yielding *N*-acyl enamine **485** in good yield and purity (Scheme 140). More recently Le Grognec and co-workers have developed the original Speckamp methodology by the use of microwave heating to generate *N*,*O*-acylaminals in faster times and in some cases bearing enolisable protons.[108] Therefore the Le Grognec methodology was trialed with imine **458** (Scheme 140). Frustratingly this methodology again afforded *N*-acyl enamine product **485**, with longer reaction times resulting in hydrolysis to afford the open chain ketone **486**.

Scheme 140 *Attempted synthesis of N,O aminal from imine **458***



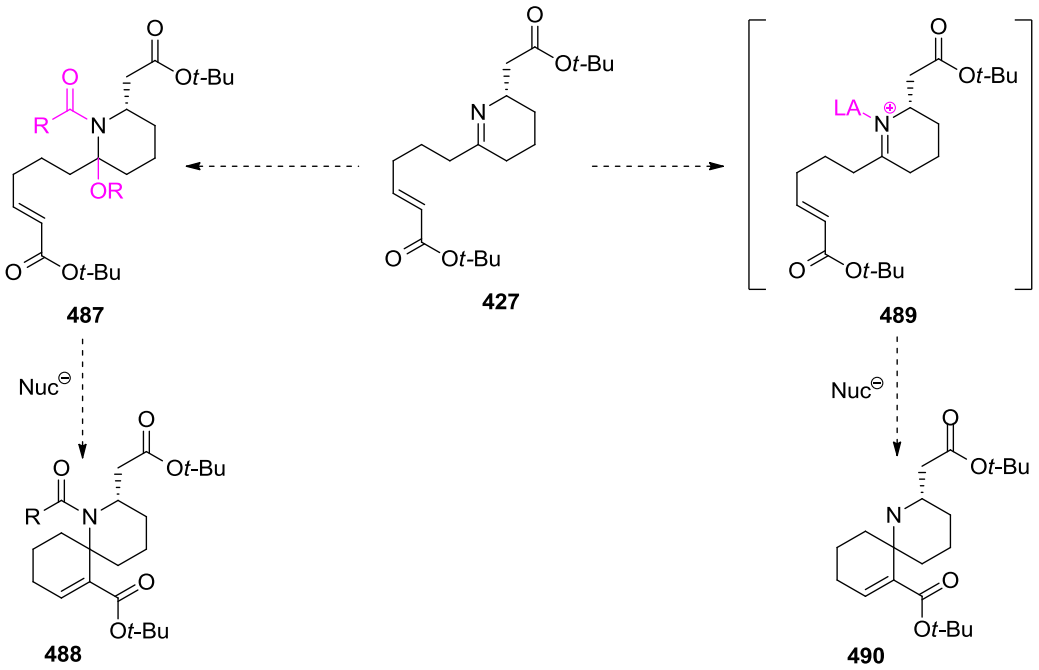
5.14 Conclusion

This chapter has described attempts towards the total synthesis of **HTX** and, whilst the synthesis is frustratingly incomplete, it is the author's belief that this chemistry is now well positioned to achieve a successful spirocyclisation reaction and elaboration of the resultant spiropiperidine intermediate to **HTX**.

A number of objectives have been achieved on the way to synthesising **HTX**: the acyclic carbon backbone of **HTX** has been synthesised; oxidation problems have been overcome by altering the protecting group strategy; the first chiral centre and amine functionality have been introduced with high enantioselectivity; and initial cyclisation of the piperidine ring of **HTX** has been realised. A positive outcome of these synthetic efforts is the discovery that *N*-trimethylsilyl-*para*-methoxybenzylamide **325** gives excellent levels of enantioselectivity for the chiral ligand **303** mediated *aza*-Michael reaction, which can be applied to the *aza*-Michael addition for a wide range of α,β-unsaturated *tert*-butyl esters. By conducting unsuccessful spirocyclisation trials using basic and Brønsted acid catalysts, the relatively low reactivity of the ketimine bond to an intramolecular nucleophilic reaction has been highlighted.

No doubt the oxidative deprotection of the nitrogen functionality can be optimised after more trials. This would give good quantities of **427** that could be used for an intra-molecular *aza*-Baylis Hillman reaction-a reaction that Aggarwal and co-workers have previously demonstrated can be used for the spirocyclisation of *N*-acyliminium ions to synthesise [4.4] spirocycles. If methodology could be developed for the synthesis of an 4° *N*,*O* aminal, it would serve as an excellent precursor for the *aza*-Baylis Hillman reaction and this methodology would undoubtedly be of great interest to the wider synthetic chemistry community (Scheme 141). Alternatively, the investigation of Lewis acid catalysis for the intramolecular Mannich reaction and the *aza*-Baylis Hillman reaction would be worthwhile and if successful would represent the first Lewis acid catalysed *aza*-Baylis Hillman reaction of a ketimine.

Scheme 141 *Future Work*



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Chapter 6 Experimental Procedures and Data

6.1 General Conditions

Reactions that required the use of dry solvents were conducted in oven dried glassware under an atmosphere of nitrogen using inert atmosphere techniques. Dry solvents were obtained by passing through anhydrous alumina columns using an Innovative Technology Inc. PS-400-7 solvent purification system. Petrol refers to the fraction of petroleum ether boiling at 40-60 °C. Hexanes refers to the hexane fraction of petroleum. Solvents were evaporated on a Büchi Rotorvapor.

^{1}H and ^{13}C NMR were run in $CDCl_3$, toluene-d_8, DMSO-d_6, methanol-d_4 and D_2O using Bruker Avance 250/300/400/500 MHz spectrometers. Chemical shifts (δ) are quoted in parts per million and are referenced to the residual solvent peak. The multiplicities and general assignments of spectroscopic data are denoted as: singlet (s), doublet (d), triplet (t), quartet (q), quintet (quint), doublet of doublets (dd), triplet of doublets (td), triplet of triplets (tt), multiplet (m), aromatic (Ph), obscured (obs.) and apparent (app.). Coupling constants (J) are quoted to the nearest 0.1 Hz. All structural assignments of both ^{1}H and ^{13}C NMR spectra were achieved with the aid of COSY, HMQC, HMBC and TOCSY experiments wherever possible and with comparisons from analogous previously reported compounds.

Infra red spectra were recorded on a Perkin Elmer Spectrum 100 FT-IR spectrometer, using a Universal ATR accessory for sampling, with only selected absorbances quoted as ν in cm^{-1}.

Mass spectra were recorded on a micrOTOF electrospray time-of-flight (ESI-TOF) mass spectrometer (Bruker Daltonik GmbH, Bremen, Germany), using acetonitrile or water to dissolve the sample.

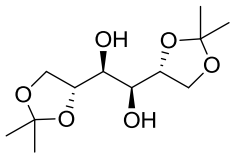
TLC was carried out using commercially available polyethylene backed plates coated with Merck Kieselgel 60 GF_{254}. Plates were visualised under UV light (at 254 nm) or by staining with potassium permanganate, ninhydrin or phosphomolybdic acid followed by heating. Flash chromatography was performed under manually generated medium pressure using Merck 60 H silica gel (35-75 μm) unless otherwise stated stated. Samples were loaded as saturated solutions in an appropriate solvent or adsorbed onto 2 mass equivalents of the column solid phase material.

ORD measurements were recorded on a Jasco J-600 spectrometer scanning from 400 nm to 204 nm using a cell with a cell length of 0.2 cm. The samples were dissolved in D_2O-MeOH (1:1) at a concentration of 6.6 mM.

All commercially available compounds were used as obtained from the chemical suppliers.

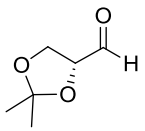
6.2 Procedures and Data for Chapter 2

1,2:5,6-Di-*O*-isopropylidene-D-mannitol[1] 180



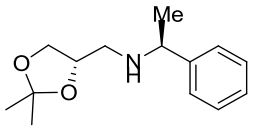
$ZnCl_2$ (125 g, 0.918 mol) was dissolved in dry acetone (770 mL) and cooled to 20 °C. D-Mannitol **179** (80 g, 0.439 mol) was added and the solution stirred at room temperature for 22 hours. K_2CO_3 (127 g, 0.919 mol) dissolved in water (130 mL) was then added to the vigorously stirred reaction mixture, which was cooled with ice/water to maintain a temperature of <5 °C. The precipitate was removed by filtration and washed with CH_2Cl_2 (2 x 200 mL). The aqueous filtrate was made basic by the addition of concentrated $NH_4OH_{(aq)}$ (1 mL), before concentration *in vacuo*. The resulting solid was dissolved in CH_2Cl_2 (500 mL), washed once with water (100 mL), dried over anhydrous $MgSO_4$, filtered and concentrated *in vacuo* to a cream solid. Recrystallisation from boiling CCl_4 afforded the title compound as white crystalline needles (61 g, 53% yield). $[\alpha]_D^{27}$ +1.9 (*c* 1.6, MeOH) (lit.[2] $[\alpha]_D^{20}$ +2.0 (*c* 2.0, MeOH)); mp. 119.5-121 °C (lit.[2] mp. 118-120 °C); ^{1}H NMR (300 MHz, $CDCl_3$) δ_H = 4.16-4.08 (2H, m, C*H*CHOH), 4.06 (2H, dd, *J* = 8.5 and 6.4 Hz, C*H*^{A}H^{B}CH), 3.90 (2H, dd, *J* = 8.5 and 5.8 Hz, CH^A*H*^{B}CH), 3.68 (2H, d, *J* = 6.2 Hz, C*H*OH), 1.35 (6H, s, C(C^A*H*$_3$)$_2$), 1.29 (6H, s, C(C^B*H*$_3$)$_2$); ^{13}C NMR (75 MHz, $CDCl_3$) δ_C = 109.4, 75.5, 71.3, 66.8, 26.7, 25.2; IR (thin film) υ_{max} (cm^{-1}): 3310 (br., OH); HRMS (ESI): *m/z* 263.1473, $C_{12}H_{23}O_6$ [M+H]$^+$ requires 263.1495.

2,3-*O*-Isopropylidene-D-glyceraldehyde[3] 181



To a suspension of **180** (5.20 g, 19.8 mmol) in CH_2Cl_2 (17 mL) was added saturated $NaHCO_{3(aq)}$ (1.9 mL). $NaIO_4$ (8.50 g, 39.6 mmol) was then added slowly, maintaining the temperature below 25 °C. The reaction mixture was stirred at room temperature and monitored by TLC (Et_2O-hexane, 2:1, R_f = 0.24). After 2 hours the suspension was filtered and the filtrate concentrated at 55 °C under atmospheric pressure. Distillation of the residue afforded the title compound as a colourless oil (3.30 g, 65% yield). $[\alpha]_D^{27}$ +60.4 (*c* 2.25, EtOAc) (lit.[4] $[\alpha]_D^{28}$ +59.7 (*c* 4.1, $CHCl_3$)); bp. 74-76 °C @ 35 Torr (lit.[5] bp. 67-73 °C @ 30 Torr); ^{1}H NMR (300 MHz, $CDCl_3$) δ_H = 9.74 (1H, d, *J* = 1.9 Hz, C*H*O), 4.41 (1H, ddd, *J* = 7.5, 4.8 and 1.8 Hz, C*H*CHO), 4.20 (1H, dd, *J* = 8.8 and 7.4 Hz, CH^AH^B), 4.12 (1H, dd, *J* = 8.8 and 4.8 Hz, CH^AH^B), 1.51 (3H, s, C$^AH_3$), 1.44 (3H, s, C$^BH_3$); ^{13}C NMR (75 MHz, $CDCl_3$) δ_C = 201.8, 111.3, 79.8, 65.6, 26.2, 25.1; IR (thin film) υ_{max} (cm^{-1}): 1734 (m, C=O); HRMS (ESI): *m/z* 131.0681, $C_6H_{11}O_3$ $[M+H]^+$ requires 131.0708.

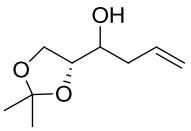
(*S*)-*N*-(((*S*)-2,2-Dimethyl-1,3-dioxolan-4-yl)methyl)-1-phenylethanamine 187



Under an inert atmosphere (*S*)-(−)-α-methylbenzylamine (0.041 ml, 0.325 mmol) was added to a solution of aldehyde **181** (21 mg, 0.163 mmol) in dry MeOH (0.2 mL) and stirred over 3Å MS for 12 h. The reaction mixture was then cooled to 0 °C before the careful addition of sodium borohydride (3 mg, 0.082 mmol). The reaction was monitored by TLC and upon complete consumption of the intermediate (CH_2Cl_2-MeOH, 20:1, R_f = 0.41) the reaction was concentrated *in vacuo*. The residue was partioned between CH_2Cl_2 and water, and the organic layer washed twice more before being dried over anhydrous $MgSO_4$ and concentrated *in vacuo* to afford the title compound as a white solid

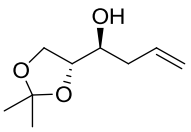
(37 mg, 96% yield). $[\alpha]_D^{30}$ −45.1 (*c* 1.2, $CDCl_3$); R_f = 0.11 (CH_2Cl_2-MeOH, 20:1); ^{1}H NMR (300 MHz, $CDCl_3$) δ_H = 7.30-7.20 (5H, m, Ph), 4.26 (1H, qd, *J* = 6.5 and 4.1 Hz, C*H*CH_2NH), 4.05 (1H, dd, *J* = 8.0 and 6.4 Hz, C*H*^{A}H^{B}CH), 3.81 (1H, q, *J* =6.6 Hz, C*H*CH_3), 3.72 (1H, dd, *J* = 7.9 and 6.6 Hz, CH^A*H*^{B}CH), 2.71 (1H, dd, *J* = 12.1 and 4.1 Hz, C*H*^{A}H^{B}NH), 2.58 (1H, dd, *J* = 12.1 and 6.8 Hz, CH^{A}C*H*^{B}NH), 1.53 (1H, br. s, N*H*), 1.32 (3H, s, CC^A*H*$_3$C^{B}H$_3$), 1.31 (3H, d, *J* = 6.6 Hz, CHC*H*$_3$), 1.27 (3H, s, CC^{A}H$_3$C^B*H*$_3$); ^{13}C NMR (75 MHz, $CDCl_3$) δ_C = 145.5, 128.5, 126.9, 126.8, 109.1, 75.3, 67.4, 58.3, 50.1, 26.9, 25.5, 24.6; IR (thin film) υ_{max} (cm^{-1}): 3026 (w, Csp^2-H), 2986 (w, Csp^3-H); HRMS (ESI): *m/z* 258.1448, $C_{14}H_{21}NNaO_2$ $[M+Na]^+$ requires 258.1470.

1-((*R*)-2,2-Dimethyl-1,3-dioxolan-4-yl)but-3-en-1-ol



To a solution of aldehyde **181** (1.00 g, 7.74 mmol) in dry THF (5 mL), cooled to 0 °C, were added zinc dust (1.01 g, 15.5 mmol) and allyl bromide (1.87 g, 15.5 mmol). Saturated $NH_4Cl_{(aq)}$ (3 mL) was then added to the reaction mixture over 10 minutes, maintaining the temperature between 0-5 °C. The reaction mixture was then allowed to warm to room temperature and monitored by TLC (EtOAc-petrol, 1:1, R_f = 0.22). After 24 hours no starting material was detected, therefore the reaction mixture was filtered through celite and the THF removed *in vacuo*. The residue was partitioned between water and CH_2Cl_2 and the aqueous layer further extracted with CH_2Cl_2 (2 x 20 mL). The combined organic extracts were then dried over anhydrous $MgSO_4$, filtered and concentrated *in vacuo* to afford the title compound as a colourless oil (0.87 g, 65% yield), which required no further purification.

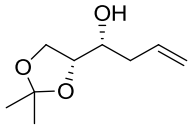
Major diastereomer[6] **182**



^{1}H NMR (300 MHz, $CDCl_3$) δ_H = 5.94-5.72 (1H, m, C*H*=CH_2), 5.20-5.05 (2H, m, CH=C*H*$_2$), 4.07-3.84 (3H, m, OC*H*$_2$-C*H*), 3.80-3.66 (1H, m, C*H*OH), 2.44-2.32 (1H, br. s, O*H*), 2.32-

2.08 (2H, m, CH_2CH=CH$_2$), 1.40 (3H, s, CC^AH_3C^{B}H$_3$), 1.33 (3H, s, CC^{A}H$_3$C^BH_3); ^{13}C NMR (75 MHz, $CDCl_3$) δ_C = 133.2, 117.1, 108.1, 77.2, 69.6, 64.4, 36.7, 25.6, 24.3.

Minor diastereomer[7] **189**

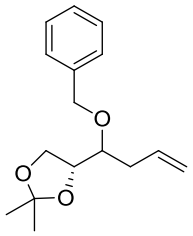


^{1}H NMR (300 MHz, $CDCl_3$) δ_H = 5.94-5.72 (1H, m, CH=CH$_2$), 5.20-5.05 (2H, m, CH=CH_2), 4.07-3.84 (3H, m, CH_2-CH), 3.63-3.51 (1H, m, CHOH), 2.57-2.46 (1H, br. s, OH), 2.32-2.08 (2H, m, CH_2CH=CH$_2$), 1.40 (3H, s, CC^AH_3C^{B}H$_3$), 1.33 (3H, s, CC^{A}H$_3$C^BH_3); ^{13}C NMR (75 MHz, $CDCl_3$) δ_C = 133.1, 116.7, 108.4, 77.6, 70.6, 65.0, 37.2, 25.6, 24.3.

Mixture of the 2 diastereomers (ratio 6 : 1):

$[\alpha]_D^{25}$ +11.8 (*c* 2.5, CH_2Cl_2); IR (thin film) υ_{max} (cm^{-1}): 3467 (br., OH); HRMS (ESI): *m/z* 195.0984, $C_9H_{16}NaO_3$ $[M+Na]^+$ requires 195.0997.

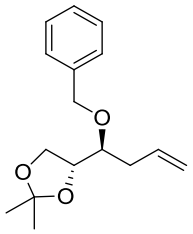
(4*R*)-4-(1-(Benzyloxy)but-3-en-1-yl)-2,2-dimethyl-1,3-dioxolane



To a solution of alcohols **182** and **189** (0.50 g, 2.90 mmol) in dry THF (3.8 mL) at 0 °C was added potassium *tert*-butoxide (0.46 g, 4.06 mmol) and the mixture stirred for 5 minutes. Benzyl bromide (0.60 g, 3.48 mmol) was then added dropwise, whilst maintaining the low temperature. When the addition was complete, the cooling bath was removed and the reaction mixture was stirred at room temperature. After 5 hours TLC analysis showed that the reaction had gone to completion, so the reaction mixture was quenched by the addition of saturated $NH_4Cl_{(aq)}$ (21 mL) and extracted with EtOAc (3 x 20 mL). The combined organic extracts were washed with water and brine, then dried over anhydrous $MgSO_4$, filtered and concentrated *in vacuo* to give a yellow oil, which was purified by silica

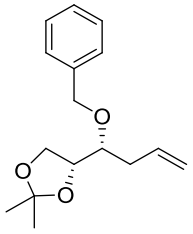
gel chromatography (petrol-EtOAc, 50:1, R_f = 0.2) to afford the title compound as a colourless oil (0.41 g, 54% yield).

Major diastereomer[8] **183**



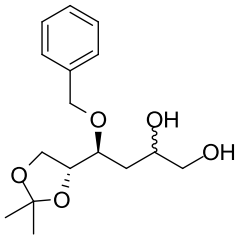
$[\alpha]_D^{26}$ +61.48 (*c* 3.5, CH_2Cl_2); ^{1}H NMR (300 MHz, $CDCl_3$) δ_H = 7.32-7.15 (5H, m, Ph), 5.92-5.73 (1H, m, C*H*=CH_2), 5.13-4.98 (2H, m, CH=C*H*$_2$), 4.59 (1H, d, *J* = 11.4 Hz, PhC*H*^{A}H^B), 4.54 (1H, d, *J* = 11.4 Hz, PhCH^A*H*^{B}), 4.08-3.92 (2H, m, OC*H*$_2$CH), 3.82 (1H, dd, *J* = 7.7 and 6.1 Hz, C*H*-CH-OBn), 3.50 (1H, app. q, *J* = 5.7 Hz, C*H*OBn), 2.44-2.19 (2H, m, C*H*$_2$CH=CH_2), 1.35 (3H, s, CC^A*H*$_3$C^{B}H$_3$), 1.28 (3H, s, CC^{A}H$_3$C^B*H*$_3$); ^{13}C NMR (75 MHz, $CDCl_3$) δ_C = 138.4, 134.2, 128.4, 127.8, 127.7, 117.5, 109.1, 78.9, 77.2, 72.5, 66.4, 35.6, 26.7, 25.4; IR (thin film) u_{max} (cm^{-1}): 1641 (w, C=C); HRMS (ESI): *m/z* 285.1442, $C_{16}H_{22}NaO_3$ $[M+Na]^+$ requires 285.1467.

Minor diastereomer[9] **190**



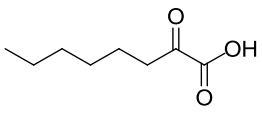
$[\alpha]_D^{26}$ +4.0 (*c* 3.1, CH_2Cl_2); ^{1}H NMR (300 MHz, $CDCl_3$) δ_H = 7.41-7.32 (5H, m, Ph), 5.94-5.83 (1H, m, C*H*=CH_2), 5.21-5.06 (2H, m, CH=C*H*$_2$), 4.73 (1H, d, *J* = 11.9 Hz, PhC*H*^{A}H^B), 4.68 (1H, d, *J* = 11.9 Hz, PhCH^A*H*^{B}), 4.23 (1H, app. q, *J* = 6.8 Hz, C*H*-CH-OBn), 4.00 (1H, dd, *J* = 8.2 and 6.7 Hz, C*H*^{A}H^{B}O), 3.73 (1H, dd, *J* = 8.2 and 7.4 Hz, CH^A*H*^{B}O), 3.53 (1H, dt, *J* = 6.8 and 4.4 Hz, C*H*OBn), 2.29-2.20 (2H, m, C*H*$_2$CH=CH_2), 1.45 (3H, s, CC^A*H*$_3$C^{B}H$_3$), 1.39 (3H, s, CC^{A}H$_3$C^B*H*$_3$); ^{13}C NMR (75 MHz, $CDCl_3$) δ_C = 138.7, 134.6, 128.3, 127.8, 127.6, 117.2, 109.3, 79.3, 77.9, 72.6, 65.8, 35.3, 26.6, 25.5; IR (thin film) u_{max} (cm^{-1}): 1642 (w, C=C); HRMS (ESI): *m/z* 285.1442, $C_{16}H_{22}NaO_3$ $[M+Na]^+$ requires 285.1467.

(4*S*)-4-(Benzyloxy)-4-((*R*)-2,2-dimethyl-1,3-dioxolan-4-yl)butane-1,2-diol 184



Under an inert atmosphere, OsO_4 (0.01 g, 0.0381 mmol) was added to a solution of the alkene **183** (0.10 g, 0.381 mmol) in acetone/water (8:1, 2.9 mL). After 5 minutes 4-methyl morpholine *N*-oxide (50 wt% aq. soln., 49 mg, 0.419 mmol) was added and the reaction mixture stirred for a further 48 hours. The reaction mixture was then concentrated *in vacuo* and the residue purified by silica gel chromatography (EtOAc, R_f = 0.47) to afford the title compound as a colourless oil (0.08 g, 71% yield). Major diastereomer[8]: ^{1}H NMR (300 MHz, $CDCl_3$) δ_H = 7.30-7.22 (5H, m, Ph), 4.69 (1H, d, *J* = 11.3 Hz, C*H*^{A}H^{B}Ph), 4.54 (1H, d, *J* = 11.3 Hz, CH^A*H*^{B}Ph), 4.17-3.96 (2H, m, C*H*$_2$O), 3.91-3.77 (2H, m, C*H*$_2$OH), 3.77-3.65 (1H, m, C*H*O), 3.57-3.46 (1H, m, C*H*OH), 3.41-3.32 (1H, m, C*H*OBn), 2.71 (1H, br. s, O*H*), 1.73-1.64 (2H, m, C*H*$_2$CHOBn), 1.38 (3H, s, CC^A*H*$_3$C^{B}H$_3$), 1.29 (3H, s, CC^A*H*$_3$C^{B}H$_3$); ^{13}C NMR (75 MHz, $CDCl_3$) δ_C = 137.7, 128.6, 128.1, 127.9, 109.3, 77.9, 77.0, 72.9, 70.2, 66.6, 66.1, 34.5, 26.4, 25.1. Minor diastereomer[8]: ^{1}H NMR (300 MHz, $CDCl_3$) δ_H = 7.30-7.22 (5H, m, Ph), 4.59 (2H, s, C*H*$_2$Ph), 4.17-3.96 (2H, m, C*H*$_2$O), 3.91-3.77 (2H, m, C*H*$_2$OH), 3.77-3.65 (1H, m, C*H*O), 3.57-3.46 (1H, m, C*H*OH), 3.41-3.32 (1H, m, C*H*OBn), 2.71 (1H, br. s, O*H*), 1.65-1.55 (2H, m, C*H*$_2$CHOBn), 1.35 (3H, s, CC^A*H*$_3$C^{B}H$_3$), 1.29 (3H, s, CC^A*H*$_3$C^{B}H$_3$); ^{13}C NMR (75 MHz, $CDCl_3$) δ_C = 138.0, 128.5, 128.1, 128.0, 109.4, 78.0, 77.7, 73.0, 68.9, 67.0, 66.5, 34.3, 26.5, 25.2. Mixture of the 2 diastereomers (ratio 1:0.9): $[\alpha]_D^{25}$ −1.7 (*c* 1.1, CH_2Cl_2); IR (thin film) υ_{max} (cm^{-1}): 3382 (br., OH); HRMS (ESI): *m/z* 295.1541, $C_{16}H_{23}O_5$ [M-H]$^-$ requires 295.1545.

2-Oxooctoanoic acid[10] 192



Method 1[11]:

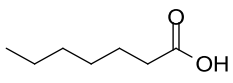
To a solution of IBX (2.30 g, 8.20 mmol) in DMSO (25 mL) was added diol **191** (0.30 g, 2.05 mmol) and the reaction was stirred at room temperature for 5 hours, whereupon TLC analysis showed that the reaction had gone to completion. The reaction mixture was diluted with water (30 mL) and extracted with Et_2O (3 x 50 mL). The combined organic extracts were dried over anhydrous $MgSO_4$, filtered and concentrated *in vacuo* to afford the title compound as a white solid (0.21 g, 65% yield).

Method 2[12]:

To a solution of octan-1,2-diol **191** (0.10 g, 0.684 mmol) in acetone (10.5 mL) was added 5% $NaHCO_{3(aq)}$ (3.6 mL). After cooling the reaction mixture to 0 °C it was treated with KBr (0.013 g, 0.137 mmol) and TEMPO (0.23 g, 1.50 mmol), followed by the dropwise addition of 0.63 M $NaClO_{(aq)}$ (2.73 ml, 1.78 mmol). After 1 hour a further portion of 0.63 M $NaClO_{(aq)}$ (1.05 ml, 0.684 mmol) was added dropwise and the solution was stirred at 0 °C for 2 hours. The reaction was then quenched by the addition of 5% $NaHCO_{3(aq)}$ (5.5 mL) and concentrated *in vacuo* to reduced volume. The basic aqueous residue was washed with Et_2O (2 x 20 mL), acidified to pH 3 by the careful addition of 2 M HCl and then extracted with EtOAc (3 x 50 mL). The combined organic extracts were washed with water and brine, then dried over anhydrous $MgSO_4$, filtered and concentrated *in vacuo* to afford the title compound as a white solid (97 mg, 90% yield).

Mp. 48-50 °C (lit.[10] mp. 50-55 °C); ^{1}H NMR (300 MHz, $CDCl_3$) δ_H = 9.76 (1H, br. s, COO*H*), 2.86 (2H, t, *J* = 7.3 Hz, C*H*$_2$C=O), 1.58 (2H, qn(is, *J* = 7.3 Hz, C*H*$_2$CH$_2$C=O), 1.34-1.18 (6H, m, C*H*$_2$C*H*$_2$C*H*$_2$), 0.82 (3H, t, *J* = 6.8 Hz, C*H*$_3$CH$_2$); ^{13}C NMR (75 MHz, $CDCl_3$) δ_C = 195.8, 160.7, 37.7, 31.4, 28.6, 23.0, 22.4, 14.0; IR (thin film) υ_{max} (cm^{-1}): 3066 (br., OH), 1702 (s, C=O); HRMS (ESI): *m/z* 157.0871, $C_8H_{13}O_3$ [M-H]$^-$ requires 157.0865.

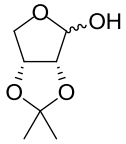
Heptanoic acid[13] 193



To a solution of octan-1,2-diol **191** (0.30 g, 2.05 mmol) in acetonitrile/water (2:1 v/v, 27 mL) was added 2-iodobenzoic acid (0.102 g, 0.41 mmol) and oxone (2.27 g, 3.69 mmol). The mixture was stirred at 70 °C for 6 hours and then cooled in an ice bath. The suspension was filtered and the solid residue washed with EtOAc (50 mL). The combined

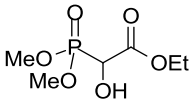
filtrates were extracted with EtOAc (100 mL) and the organic phase was washed with water (2 x 50 mL), dried over anhydrous $MgSO_4$, filtered and concentrated *in vacuo* to afford the title compound as a yellow oil (0.23 g, 71% yield). ^{1}H NMR (300 MHz, $CDCl_3$) δ_H = 11.2 (1H, br. s, COO*H*), 2.28 (2H, t, *J* = 7.5 Hz, C*H*$_2$C=O), 1.58 (2H, quintet, *J* = 7.5 Hz, C*H*$_2$CH$_2$C=O), 1.35-1.16 (6H, m, C*H*$_2$C*H*$_2$C*H*$_2$), 0.82 (3H, t, *J* = 6.8 Hz, C*H*$_3$CH$_2$); ^{13}C NMR (75 MHz, $CDCl_3$) δ_C = 180.5, 34.1, 31.4, 28.7, 24.6, 22.5, 14.0; IR (thin film) υ_{max} (cm^{-1}): 2951 (br., OH), 1707 (s, C=O); HRMS (ESI): *m/z* 129.0900, $C_7H_{13}O_2$ [M-H]$^-$ requires 129.0916.

(3a*S*,6a*R*)-2,2-Dimethyltetrahydrofuro[3,4-d][1,3]dioxol-4-ol[14] **198**



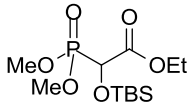
To a solution of (-)-2,3-*O*-isopropylidine-D-erythronolactone **197** (4.73 g, 29.9 mmol) in dry CH_2Cl_2 (85 mL) at -78 °C and under inert atmosphere, a solution of DIBAL (1.0 M in hexane, 34.3 ml, 34.4 mmol) was added dropwise. After stirring the mixture at reduced temperature for 2 hours, TLC (EtOAc-toluene, 1:1) showed the reaction had gone to completion. MeOH (5 mL) was added dropwise and the mixture was then poured, with care, into a rapidly stirred ice/water mixture (75 mL). The suspension was filtered through celite and the filtered solids washed well with Et_2O. The combined filtrates were dried over anhydrous $MgSO_4$, filtered and concentrated *in vacuo* to afford the title compound as a colourless oil which required no further purification (3.11 g, 65% yield). $[\alpha]_D^{20}$ +75.0 (*c* 1.12, $CHCl_3$); R_f = 0.47 (EtOAc-toluene, 1:1); ^{1}H NMR (300 MHz, $CDCl_3$) δ_H = 5.35 (1H, b, C*H*OH), 4.77 (1H, dd, *J* = 5.8 and 3.2 Hz, C*H*CH$_2$), 4.51 (1H, d, *J* = 5.8 Hz, C*H*CHOH), 4.01 (1H, dd, *J* = 10.4 and 3.4 Hz, C*H*^{A}H^B), 3.95 (1H, d, *J* = 10.4 Hz, CH^A*H*^{B}), 2.74 (1H, br. s, O*H*), 1.40 (3H, s, CC^A*H*$_3$C^{B}H$_3$), 1.25 (3H, s, CC^{A}H$_3$C^B*H*$_3$); ^{13}C NMR (75 MHz, $CDCl_3$) δ_C = 112.3, 101.7, 85.2, 79.9, 71.8, 26.2, 24.7; IR (thin film) υ_{max} (cm^{-1}): 3421 (br., OH); HRMS (ESI): *m/z* 183.0629, $C_7H_{12}NaO_4$ [M+Na]$^+$ requires 183.0633.

Ethyl 2-(dimethoxyphosphoryl)-2-hydroxyacetate[15] 204



Ethyl glyoxalate (48 ml of a ~50 wt% toluene solution, 0.230 moles) was cracked at reflux for 1 hour. On cooling to room temperature, toluene (260 mL), dimethyl phosphate (26.4 ml, 0.200 moles) and *para*-toluenesulfonic acid monohydrate (0.140 g, 0.740 mmol) were added sequentially and the reaction mixture was heated at reflux for 16 hours. After cooling, the solution was concentrated *in vacuo* to a yellow oil, which was purified by silica gel chromatography (CH_2Cl_2-MeOH, 50:1, R_f = 0.2) to afford the title compound as a colourless oil (28.3 g, 71% yield). ^{1}H NMR (300 MHz, $CDCl_3$) δ_H = 4.56 (1H, d, *J* = 16.2 Hz, C*H*OH), 4.36-4.24 (2H, m, CH_2CH$_3$), 3.84 (3H, d, *J* = 3.9 Hz, OC^AH_3), 3.80 (3H, d, *J* = 3.9 Hz, OC^BH_3), 1.29 (3H, t, *J* = 7.2 Hz, CH$_2$CH_3); ^{13}C NMR (75 MHz, $CDCl_3$) δ_C = 169.2 (d, *J* = 1.8 Hz), 68.7 (d, *J* = 155.1 Hz), 62.7, 54.3 (d, *J* = 6.3 Hz), 54.0 (d, *J* = 6.3 Hz), 14.0; ^{31}P (122 MHz, $CDCl_3$) δ_P = 19.0; IR (thin film) υ_{max} (cm^{-1}): 3229 (br., OH), 1752 (s, C=O); HRMS (ESI): *m/z* 213.0531, $C_6H_{14}O_6P$ $[M+H]^+$ requires 213.0528.

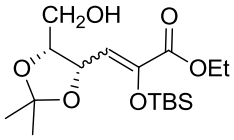
Ethyl 2-((*tert*-butyldimethylsilyl)oxy)-2-(dimethoxyphosphoryl)acetate[16] 1



To a stirred solution of **204** (19.1 g, 0.09 moles) in CH_2Cl_2 (1.4 L) was added imidazole (18.4 g, 0.271 moles), DMAP (1.65 g, 0.014 moles) and *tert*-butyldimethylsilyl chloride (27.2 g, 0.181 moles). The mixture was stirred at room temperature for 18 hours when TLC analysis (EtOAc-petrol, 1:1) showed that the reaction had gone to completion. The reaction mixture was washed with saturated $NaHCO_{3(aq)}$, dried over anhydrous $MgSO_4$, filtered and concentrated *in vacuo*. The residue was purified by silica gel chromatography (EtOAc-petrol, 1:2-1:1), to afford the title compound as a colourless oil (24 g, 81% yield). R_f = 0.35 (EtOAc-petrol, 1:1); ^{1}H NMR (300 MHz, $CDCl_3$) δ_H = 4.51 (1H, d, *J* = 18.6 Hz, C*H*OSi), 4.23-4.11 (2H, m, CH_2CH$_3$), 3.75 (3H, d, *J* = 4.9 Hz, OC^AH_3), 3.71 (3H, d, *J* = 4.9 Hz, OC^BH_3), 1.20 (3H, t, *J* = 7.2 Hz, CH$_2$CH_3), 0.82 (9H, s, C(CH_3)$_3$), 0.01 (6H, s,

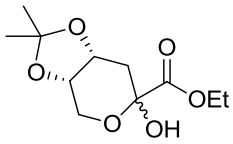
Si(CH_3)$_2$); ^{13}C NMR (75 MHz, $CDCl_3$) δ_C = 168.4 (d, *J* = 2.5 Hz), 70.6 (d, *J* = 161 Hz), 61.9, 54.2 (d, *J* = 4.4 Hz), 54.1 (d, *J* = 4.5 Hz), 25.5, 18.4, 14.1, -5.4 (d, *J* = 12.2 Hz); ^{31}P (122 MHz, $CDCl_3$) δ_P = 18.9; IR (thin film) υ_{max} (cm^{-1}): 1753 (m, C=O); HRMS (ESI): *m/z* 327.1376, $C_{12}H_{28}O_6PSi$ [M+H]$^+$ requires 327.1393.

(4*S*, 5*R*)-Ethyl 2-((*tert*-butyldimethylsilyl)oxy)-3-(5-(hydroxymethyl)-2,2-dimethyl-1,3-dioxolan-4-yl)acrylate 199



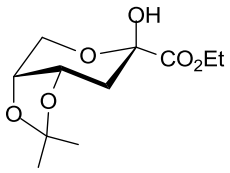
A dry THF (24 mL) solution of LHMDS (1.0 M in THF, 21.5 ml, 21.5 mmol) was cooled to -78 °C. Ethyl 2-((*tert*-butyldimethy-lsilyl)oxy)-2-(dimethoxyphosphoryl)acetate **1** (6.20 g, 19.0 mmol) was dissolved in dry THF (24 mL) and the solution added dropwise. The reaction mixture was stirred for a further 10 minutes following the addition. A THF (24 mL) solution of (3a*S*,6a*R*)-2,2-dimethyltetrahydrofuro[3,4-d][1,3]dioxol-4-ol **198** (3.04 g, 19.0 mmol) was then added dropwise. The reaction mixture was then stirred for another 5 minutes before being allowed to warm to 0 °C. Once TLC (EtOAc-toluene, 1:1) confirmed the reaction had gone to completion, the suspension was poured into Et_2O (290 mL), washed sequentially with 1 M $HCl_{(aq)}$ and saturated $NaHCO_{3(aq)}$, dried over anhydrous $MgSO_4$, filtered and concentrated *in vacuo*. The residue was purified by silica gel chromatography (EtOAc-petrol, 1:20 to 1:1) to give the title compound as a colourless oil (4.58 g, 67% yield). $[\alpha]_D^{25}$ +31.3 (*c* 1.5, $CHCl_3$); ^{1}H NMR (300 MHz, $CDCl_3$) δ_H = 5.57 (1H, d, *J* = 7.8 Hz, C=C*H*), 5.48-5.43 (1H, m, C*H*CH=C), 4.50 (1H, td, *J* = 6.4 and 4.2 Hz, C*H*CH_2OH), 4.32-4.23 (2H, m, OC$H_2$$CH_3$), 3.65 (1H, dd, *J* = 11.2 and 4.3 Hz, HOC$H^A$$H^B$), 3.57 (1H, dd, *J* = 11.2 and 6.2 Hz, HOCH^AH^B), 2.08 (1H, br. s, O*H*), 1.56 (3H, s, CC^AH_3C^BH_3), 1.44 (3H, s, CC^AH_3C^BH_3), 1.37 (3H, t, *J* = 7.2 Hz, OCH_2CH_3), 0.99 (9H, s, C(CH_3)$_3$, 0.21 (6H, s, Si(CH_3)$_2$); ^{13}C NMR (75 MHz, $CDCl_3$) δ_C = 164.5, 142.2, 121.2, 115.6, 109.0, 108.6, 78.71, 73.8, 62.2, 61.4, 27.7, 25.8, 25.5, 25.0, 18.2, 4.2, -4.8, -4.9; IR (thin film) υ_{max} (cm^{-1}): 3492 (br., OH), 1726 (m, C=O), 1641 (w, C=C); HRMS (ESI): *m/z* 383.1877, $C_{17}H_{32}NaO_6Si$ [M+Na]$^+$ requires 383.1866.

(3a*S*,7a*R*)-Ethyl 6-hydroxy-2,2-dimethyltetrahydro-3aH-[1,3]dioxolo[4,5-c]pyran-6-carboxylate 200



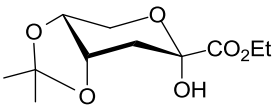
In oven-dried glassware under inert atmosphere CsF (0.819 g, 5.39 mmol) and AcOH (0.770 ml, 13.5 mmol) were added to a solution of **199** (0.970 g, 2.69 mmol) in dry MeCN (97 mL) at 0 °C. The resulting mixture was stirred at 0 °C for a further 30 minutes and then at room temperature. After 30 minutes TLC analysis (EtOAc-petrol, 1:2) showed complete consumption of starting material **199**, so the reaction mixture was diluted with EtOAc (300 mL) and petrol (300 mL), washed with saturated $NaHCO_{3(aq)}$, dried over anhydrous $MgSO_4$, filtered and concentrated *in vacuo* to afford the title compound as a yellow oil (0.603 g, 91% yield).

Major isomer β-**200**



^{1}H NMR (300 MHz, $CDCl_3$) δ_H = 4.42-4.33 (1H, m, C*H*CH_2COH), 4.22-4.12 (2H, m, OC*H*$_2$$CH_3$), 4.09-4.05 (1H, m, C*H*CH$_2$COH), 4.03 (1H, dd, *J* = 12.5 and 2.9 Hz, OC*H*^{A}H^B), 3.94 (1H, br. s, O*H*), 3.90 (1H, dd, *J* = 12.5 and 2.0 Hz, OCH^A*H*^{B}), 2.21 (1H, dd, *J* = 13.6 and 8.2 Hz, C*H*^{A}H^{B}COH), 1.93 (1H, dd, *J* = 13.6 and 6.1 Hz, CH^A*H*^{B}COH), 1.40 (3H, s, CC^A*H*$_3$C^{B}H$_3$), 1.24 (3H, s, CC^{A}H$_3$C^B*H*$_3$), 1.22 (3H, t, *J* = 7.1 Hz, OCH$_2$C*H*$_3$); ^{13}C NMR (75 MHz, $CDCl_3$) δ_C = 169.9, 108.8, 94.3, 71.2, 69.2, 62.5, 61.6, 33.2, 27.6, 25.8, 14.0.

Minor isomer α-**200**



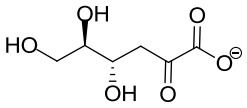
^{1}H NMR (300 MHz, $CDCl_3$) δ_H = 4.50-4.41 (1H, m, C*H*CH_2COH), 4.24-4.11 (3H, m, C*H*CHCH$_2$COH, OC*H*$_2$$CH_3$), 3.80 (1H, dd, *J* = 12.4 and 5.9 Hz, OC*H*^{A}H^B), 3.72 (1H, dd, *J* = 12.4 and 4.9 Hz, OCH^A*H*^{B}), 2.33 (1H, dd, *J* = 15.1 and 4.4 Hz, C*H*^{A}H^{B}COH), 2.08 (1H,

dd, *J* = 15.1 and 5.4 Hz, $CH^{A}H^{B}COH$), 1.44 (3H, s, $CC^{A}H_3C^{B}H_3$), 1.26 (3H, s, $CC^{A}H_3C^{B}H_3$), 1.23 (3H, t, *J* = 7.1 Hz, OCH_2CH_3); ^{13}C NMR (75 MHz, $CDCl_3$) δ_C = 169.5, 109.4, 94.2, 70.9, 70.6, 62.6, 62.3, 32.2, 27.2, 25.0, 14.0.

Mixture of the 2 isomers (ratio 3:1):

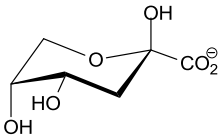
$[\alpha]_D^{23}$ +38.8 (*c* 1.60, CH_2Cl_2); R_f = 0.32 (EtOAc-petrol, 1:2); IR (thin film) υ_{max} (cm^{-1}): 3429 (br., OH), 1743 (m, C=O); HRMS (ESI): *m/z* 269.0996, $C_{11}H_{18}NaO_6$ $[M+Na]^+$ requires 269.1001.

(4*S*,5*R*)-4,5,6-Trihydroxy-2-oxohexanoic acid[17-18] **D-KDG**



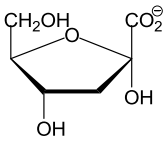
Preparation from **200** employing method 2 used for the synthesis of **D-KDX** afforded the title compound as a yellow viscous oil (0.59 g, 85% yield).

β-pyranose (45%)



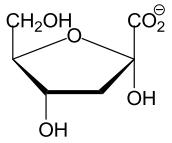
^{1}H NMR (500 MHz, $CDCl_3$) δ_H = 4.04-3.98 (1H, m, $CHOHCH_2COC$), 3.93 (1H, d, *J* = 12.7 Hz, $CH^{A}H^{B}OC$), 3.79-3.77 (1H, m, $CHOHCH_2OC$), 3.72 (1H, dd, *J* = 12.7 and 2.4 Hz, $CH^{A}H^{B}OC$), 1.95 (1H, dd, *J* = 13.2 and 12.2 Hz, $CH^{A}H^{B}COC$), 1.82 (1H, dd, *J* = 13.5 and 5.0 Hz, $CH^{A}H^{B}COC$).

α-furanose (22%)



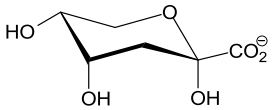
^{1}H NMR (500 MHz, $CDCl_3$) δ_H = 4.32-4.27 (1H, m, $CHOH$), 4.14-4.11 (1H, q, *J* = 4.1 Hz, $CHCH_2OH$), 3.70-3.67 (1H, m, $CH^{A}H^{B}OH$), 3.61-3.57 (1H, m, $CH^{A}H^{B}OH$), 2.48 (1H, dd, *J* = 14.0 and 7.2 Hz, $CH^{A}H^{B}$), 2.00-1.95 (1H, m, $CH^{A}H^{B}$).

β-furanose (20%)



^{1}H NMR (500 MHz, $CDCl_3$) δ_H = 4.32-4.27 (1H, m, C*H*OH), 4.00-3.95 (1H, m, C*H*CH_2OH), 3.69-3.65 (1H, m, C*H*^{A}H^{B}OH), 3.60-3.56 (1H, m, CH^A*H*^{B}OH), 2.29 (1H, dd, *J* = 13.3 and 6.7 Hz, C*H*^{A}H^B), 2.24 (1H, dd, *J* = 13.1 and 6.5 Hz, CH^A*H*^{B}).

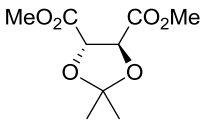
α-pyranose (12%)



^{1}H NMR (500 MHz, $CDCl_3$) δ_H = 3.86-3.81 (1H, m, C*H*OHCH$_2$COC), 2.11-2.05 (1H, m, C*H*^{A}H^{B}COC), 1.74-1.67 (CH^A*H*^{B}COC).

Equilibrium mixture of 4 isomers: $[\alpha]_D^{25}$ -16.8 (*c* 0.24, D_2O at pH 6); ^{13}C NMR (126 MHz, $CDCl_3$) δ_C = 176.5, 176.3, 176.2, 103.7, 103.1, 96.3, 86.7, 86.6, 71.4, 71.0, 67.3, 64.9, 64.8, 64.4, 62.2, 61.6, 44.0, 43.3, 33.8; I.R. (thin film) u_{max} (cm^{-1}): 1740 (m, C=O); HRMS (ESI): *m/z* 177.0402, $C_6H_9O_6$ [M-H]$^-$ requires 177.0399.

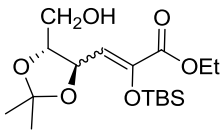
(4*S*,5*S*)-Dimethyl 2,2-dimethyl-1,3-dioxolane-4,5-dicarboxylate[19] 212



Under inert atmosphere a solution of D-tartaric acid (5.20 g, 34.6 mmol), 2,2-dimethoxypropane (9.80 ml, 79.6 mmol), MeOH (2.1 mL) and *p*-TSA (0.02 g, 0.107 mmol) were heated at 100 °C for 2.5 hours. Additional 2,2-dimethoxypropane (4.9 ml, 39.4 mmol) and cyclohexane (23 mL) were added, a distillation head was attached to the flask and *ca*. 30 ml of liquid was removed by distillation. After cooling to room temperature, K_2CO_3 (41 mg, 0.37 mmol) was added and the mixture stirred until the reddish colour abated. The reaction mixture was then concentrated *in vacuo* and purified by silica gel chromatography (EtOAc-petrol, 3:10, R_f = 0.28) to give the title compound as a colourless oil (4.98 g, 66% yield). $[\alpha]_D^{26}$ = +41.4 (c = 1.62 in $CDCl_3$); ^{1}H NMR (300 MHz, $CDCl_3$) δ_H =

4.66 (2H, s, C*H*C*H*), 3.68 (6H, s, CO_2CH_3), 1.35 (6H, s, $C(CH_3)_2$); ^{13}C NMR (75 MHz, $CDCl_3$) δ_C = 170.1, 113.9, 112.2, 77.0, 52.8, 26.7; I.R. (thin film) u_{max} (cm^{-1}): 1740 (m, C=O); HRMS (ESI): *m/z* 219.0872, $C_9H_{15}O_6$ $[M+H]^+$ requires 219.0869.

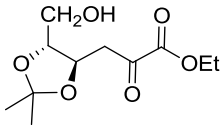
(4*S*, 5*R*)-Ethyl 2-((*tert*-butyldimethylsilyl)oxy)-3-(5-(hydroxymethyl)-2,2-dimethyl-1,3-dioxolan-4-yl)acrylate 218



To a solution of **212** (0.930 g, 4.25 mmol) in dry toluene (17 mL), under an inert atmosphere, was added DIBAL (1.0 M in toluene, 8.50 ml, 8.50 mmol) dropwise over 2 hours, maintaining the temperature at -5 – 0 °C throughout. The mixture was then stirred at 0 °C for 1 hour before being cooled to -78 °C and a further portion of DIBAL (1 M in toluene, 4.25 ml, 4.25 mmol) was then added dropwise over 1 hour. In a separate vessel, under an inert atmosphere, LHMDS (1 M in THF, 6.37 ml, 6.37 mmol) was added dropwise to a dry THF (19 mL) solution of ethyl 2-((*tert*-butyldimethylsilyl)oxy)-2-(dimethoxyphosphoryl)acetate **1** (2.08 g, 6.37 mmol) at -78 °C. After stirring this solution for 10 minutes it was added *via* cannular to the reduced mixture of **212**. The reaction mixture was stir-warmed to room temperature and stirred for a further 5 hours before being quenched with a saturated aq. soln. of potassium sodium tartrate (8.6 mL). The suspension was stirred vigorously for 2 hours, diluted with water (9 mL) and then filtered through celite. The filtrate was extracted with Et_2O (3 x 50 mL), the combined organic extracts dried over anhydrous $MgSO_4$, filtered and concentrated *in vacuo*. Purification of the residue by silica gel chromatography (EtOAc-petrol, 1:20 to 1:10) afforded the title compound as a colourless oil (0.552 g, 36% yield). Major isomer: $[\alpha]_D^{25}$ +20.0 (*c* 0.6, $CDCl_3$); R_f = 0.41 (EtOAc-petrol 3:10); ^{1}H NMR (300 MHz, $CDCl_3$) δ_H = 5.26 (1H, d, *J* = 9.0 Hz, C=C*H*), 4.99 (1H, t, *J* = 8.1 Hz, C*H*CH=C), 4.08 (2H, q, *J* = 7.2 Hz, OCH_2CH_3), 3.72-3.51 (3H, m, C*H*CH_2OH, HOCH_2), 2.60 (1H, br. s, O*H*), 1.28 (3H, s, $CC^AH_3C^BH_3$), 1.27 (3H, s, $CC^AH_3C^BH_3$), 1.16 (3H, t, *J* = 7.2 Hz, OCH_2CH_3), 0.78 (9H, s, $C(CH_3)_3$), 0.00 (6H, s, $Si(CH_3)_2$); ^{13}C NMR (75 MHz, $CDCl_3$) δ_C = 165.0, 143.4, 121.2, 109.1, 81.4, 73.2, 61.7, 61.4, 27.1, 27.0, 25.5, 18.2, 14.1, -4.7, -4.9; IR (thin film) u_{max} (cm^{-1}): 3512 (br., OH), 1722 (m, C=O); HRMS (ESI): *m/z* 383.1872, $C_{17}H_{32}O_6NaSi$ $[M+Na]^+$ requires 383.1866. Minor Isomer: $[\alpha]_D^{25}$ -8.0 (*c* 0.25, $CDCl_3$); R_f = 0.48 (EtOAc-petrol, 3:10); ^{1}H NMR (300 MHz,

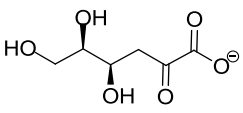
$CDCl_3$) δ_H = 5.77 (1H, d, *J* = 8.7 Hz, C=C*H*), 4.69 (1H, t, *J* = 8.7 Hz, C*H*CH=C), 4.11-3.98 (2H, m, OCH_2CH$_3$), 3.70-3.65 (1H, m, C*H*CH$_2$OH), 3.62 (1H, dd, *J* = 11.7 and 3.4, CH^AH^{B}OH), 3.62 (1H, dd, *J* = 11.7 and 4.1, CH^AH^BOH), 2.45 (1H, br. s, O*H*), 1.28 (3H, s, CC^AH_3C^{B}H$_3$), 1.26 (3H, s, CC^{A}H$_3$C^BH_3), 1.14 (3H, t, *J* = 7.2 Hz, OCH$_2$CH_3), 0.79 (9H, s, C(CH_3)$_3$, 0.05 (6H, s, Si(CH_3)$_2$); ^{13}C NMR (75 MHz, $CDCl_3$) δ_C = 165.1, 144.5, 116.7, 109.6, 81.3, 71.7, 61.5, 61.4, 27.1, 26.9, 25.8, 18.6, 14.2, -4.2, -4.4; IR (thin film) υ_{max} (cm^{-1}): 3500 (br., OH), 1727 (m, C=O); HRMS (ESI): *m/z* 383.1874, $C_{17}H_{32}NaO_6Si$ $[M+Na]^+$ requires 383.1866.

Ethyl 3-((4*R*,5*R*)-5-(hydroxymethyl)-2,2-dimethyl-1,3-dioxolan-4-yl)-2-oxopropanoate 219



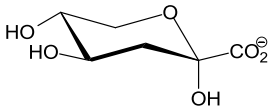
Preparation from **218** using the same method as that used for **200** afforded the title compound as a colourless oil (0.82 g, 85% yield). $[\alpha]_D^{23}$ +10.5 (*c* 2.00, $CDCl_3$); ^{1}H NMR (300 MHz, $CDCl_3$) δ_H = 4.36-4.22 (3H, m, C*H*OHCH$_2$CO, OCH_2CH$_3$), 3.82-3.77 (1H, m, C*H*OHCH$_2$OH), 3.74 (1H, dd, *J* = 11.7 and 4.0 Hz, CH^AH^{B}OH), 3.64 (1H, dd, *J* = 11.7 and 4.4 Hz, CH^AH^BOH), 3.17 (1H, dd, *J* = 17.2 and 6.9 Hz, CH^AH^{B}CO), 3.06 (1H, dd, *J* = 17.2 and 5.1 Hz, CH^AH^BCO), 2.35 (1H, br. s, O*H*), 1.34 (3H, s, CC^AH_3C^{B}H$_3$), 1.33 (3H, s, CC^{A}H$_3$C^BH_3), 1.30 (3H, t, *J* = 7.1 Hz, OCH$_2$CH_3); ^{13}C NMR (75 MHz, $CDCl_3$) δ_C = 191.6, 160.6, 109.5, 81.0, 72.6, 62.7, 61.8, 42.7, 27.03, 26.96, 13.9; IR (thin film) υ_{max} (cm^{-1}): 3471 (br., OH), 1728 (m, C=O); HRMS (ESI): *m/z* 269.0988, $C_{11}H_{18}NaO_6$ $[M+Na]^+$ requires 269.0996.

(4*R*,5*R*)-4,5,6-Trihydroxy-2-oxohexanoic acid[17-18] **D-KDGal**



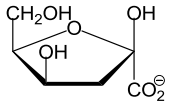
Preparation from **219** employing method 2 used for the synthesis of **D-KDX** afforded the title compound as a yellow viscous oil (0.32 g, 80% yield).

α-Pyranose (72%)



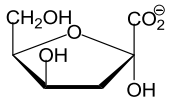
^{1}H NMR (500 MHz, $CDCl_3$) δ_H = 3.81-3.74 (1H, m, CH_2CHOH), 3.73-3.70 (1H, m, OCH^AH^B), 3.54-3.50 (2H, m, OCH^AH^BCHOH), 2.08 (1H, dd, *J* = 13.1, 5.2 Hz, CH^AH^B), 1.71 (1H, dd, *J* = 11.5, 13.1 Hz, CH^AH^B); ^{13}C NMR (126 MHz, $CDCl_3$) δ_C = 176.2, 98.5, 70.6, 68.9, 62.9, 38.9.

β-furanose (11%)



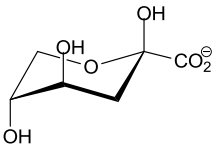
^{1}H NMR (500 MHz, $CDCl_3$) δ_H = 3.98 (1H, dd, *J* = 12.3, 3.8 Hz, $HOCH^AH^B$), 3.78-3.73 (1H, m, CH_2CHOH), 3.53-3.49 (1H, m, $OCHCH_2OH$), 3.38 (1H, dd, *J* = 12.2, 6.9 Hz, $HOCH^AH^B$), 2.38 (1H, dd, *J* = 13.6, 4.3 Hz, CH^AH^B), 1.59 (1H, dd, *J* = 13.6, 8.0 Hz, CH^AH^B).

(9%) α-furanose



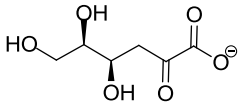
^{1}H NMR (500 MHz, $CDCl_3$) δ_H = 4.40-4.37 (1H, m, CH_2CHOH), 4.22-4.18 (1H, m, $OCHCH_2OH$), 3.79-3.75 (1H, m, $HOCH^AH^B$), 3.71-3.68 (1H, m, $HOCH^AH^B$), 2.28 (2H, d, *J* = 4.0 Hz, CH_2).

β-pyranose (5%)



^{1}H NMR (500 MHz, $CDCl_3$) δ_H = 4.47-4.43 (1H, m, CH_2CHOH), 4.13-4.09 (1H, m, OCH^AH^BCHOH), 3.70-3.65 (1H, m, OCH^AH^B), 3.57-3.53 (1H, m, OCH^AH^B), 2.47 (1H, dd, *J* = 14.4, 5.6 Hz, CH^AH^B), 2.05-2.01 (1H, m, CH^AH^B).

Acyclic (3%)

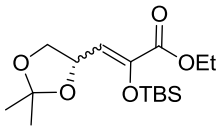


^{1}H NMR (500 MHz, $CDCl_3$) δ_H = 4.46-4.31 (1H, m, C*H*CH_2OH), 4,13-4.09 (1H, m, CH_2C*H*OH), 3.83-3.73 (2H, m, C*H*$_2$OH), 2.93 (2H, d, *J* = 6.7 Hz, C*H*$_2$).

Equilibrium mixture of 5 isomers: $[\alpha]_D^{25}$ -5.5 (*c* 3.6, MeOH-H_2O 1:1); IR (thin film) u_{max} (cm^{-1}): 1735 (m, C=O); HRMS (ESI): *m/z* 177.0405, $C_6H_9O_6$ $[M-H]^-$ requires 177.0399.

6.3 Procedures and Data for Chapter 3

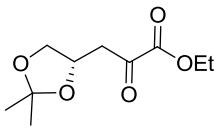
(*S*)-Ethyl 2-((*tert*-butyldimethylsilyl)oxy)-3-(2,2-dimethyl-1,3-dioxolan-4-yl)acrylate[20] 222



Preparation from aldehyde **181** using the same method as that used for the synthesis of **199** afforded, after purification by silica gel chromatography (EtOAc-petrol, 1:25), the title compound as a colourless oil (0.84 g, 73% yield). Major isomer: $[\alpha]_D^{25}$ +15.8 (*c* 3.35, $CDCl_3$); R_f = 0.42 (EtOAc-petrol, 1:20); ^{1}H NMR (300 MHz, $CDCl_3$) δ_H = 5.39 (1H, d, *J* = 7.5 Hz, C*H*=C), 5.17 (1H, app.q, *J* = 6.9 Hz, C*H*CH=C), 4.12 (1H, dd, *J* = 8.1 and 6.5 Hz, C*H*^{A}H^{B}CH), 4.07 (2H, q, *J* = 7.1 Hz, OC*H*$_2$), 3.42 (1H, dd, *J* = 8.1 and 6.8 Hz, CH^A*H*^{B}CH), 1.29 (3H, s, CC^A*H*$_3$), 1.23 (3H, s, CC^B*H*$_3$), 1.16 (3H, t, *J* = 7.2 Hz, CH_2C*H*$_3$), 0.79 (9H, s, C(C*H*$_3$)$_3$), 0.00 (6H, s, Si(C*H*$_3$)$_2$); ^{13}C NMR (75 MHz, $CDCl_3$) δ_C = 164.2, 142.0, 123.8, 109.3, 72.5, 70.0, 61.2, 26.7, 25.6, 18.2, 14.1, -4.8, -4.9. Minor isomer: R_f = 0.51 (EtOAc-petrol, 1:20); ^{1}H NMR (300 MHz, $CDCl_3$) δ_H = 5.81 (1H, d, *J* = 8.6 Hz, C*H*=C), 4.92-4.82 (1H, m, C*H*CH=C), 4.06 (2H, q, *J* = 7.0 Hz, OC*H*$_2$), 3.97 (1H, dd, *J* = 8.1 and 6.2 Hz, C*H*^{A}H^{B}CH) 3.42 (1H, dd, *J* = 8.1 and 6.7 Hz, CH^A*H*^{B}CH), 1.29 (3H, s, CC^A*H*$_3$), 1.24 (3H, s, CC^B*H*$_3$), 1.15 (3H, t, *J* = 7.2 Hz, CH_2C*H*$_3$), 0.80 (9H, s, C(C*H*$_3$)$_3$), 0.03 (6H, s, Si(C*H*$_3$)$_2$); IR

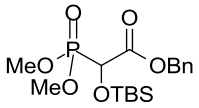
(thin film) υ_{max} (cm^{-1}): 1720 (m, C=O); HRMS (ESI): *m/z* 353.1739, $C_{16}H_{30}NaO_5Si$ $[M+Na]^+$ requires 353.1760.

(*S*)-Ethyl 3-(2,2-dimethyl-1,3-dioxolan-4-yl)-2-oxopropanoate 223



Preparation from **222** using the same method as that used for **200** afforded the title compound as a yellow oil (0.54 g, 86% yield). $[\alpha]_D^{30}$ +12 (*c* 1.25, CH_2Cl_2); ^{1}H NMR (300 MHz, $CDCl_3$) δ_H = 4.45 (1H, quintet, *J* = 6.4 Hz, CH_2CHCH_2), 4.24 (2H, q, *J* = 7.2 Hz, OCH_2CH_3), 4.10 (1H, dd, *J* = 8.2 and 6.1 Hz, $CH^AH^BOC(CH_3)_2$), 3.52 (1H, dd, *J* = 8.5 and 6.3 Hz, $CH^AH^BOC(CH_3)_2$), 3.21 (1H, dd, *J* = 17.6 and 6.1 Hz, $CH^AH^BC=O$), 2.92 (1H, dd, *J* = 17.6 and 6.8 Hz, $CH^AH^BC=O$), 1.32 (3H, s, CC^AH_3), 1.28 (3H, t, *J* = 7.2 Hz, OCH_2CH_3), 1.26 (3H, s, CC^BH_3); ^{13}C NMR (75 MHz, $CDCl_3$) δ_C = 192.0, 160.4, 109.3, 71.1, 69.1, 62.7, 43.8, 26.8, 25.5, 14.0; IR (thin film) υ_{max} (cm^{-1}): 1729 (m, C=O); HRMS (ESI): *m/z* 239.0881, $C_{10}H_{16}O_5Na$ $[M+Na]^+$ requires 239.0895.

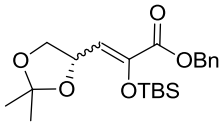
Benzyl 2-((*tert*-butyldimethylsilyl)oxy)-2-(dimethoxyphosphoryl)acetate 228



Titanium tetraisopropoxide (0.58 ml, 1.90 mmol) was added to a solution of **1** (1.80 g, 5.52 g) in benzyl alcohol (36 mL) under inert atmosphere. The reaction mixture was heated at 100 °C for 12 hours, at which point TLC analysis (EtOAc-petrol, 1:10) showed complete consumption of **1**. The reaction mixture was cooled to room temperature and quenched with 1 M $HCl_{(aq)}$ (90 mL). The reaction mixture was filtered through celite and then extracted with a solution of pentane/Et_2O (1:1) (3 x 100 mL). The combined organic extracts were dried over anhydrous $MgSO_4$, filtered and concentrated *in vacuo*. The residue was purified by silica gel chromatography (EtOAc-petrol, 1:5, R_f = 0.32) affording

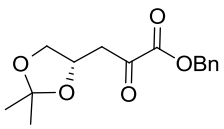
the title compound as a colourless oil (0.65 g, 30% yield). ^{1}H NMR (300 MHz, $CDCl_3$) δ_H = 7.41-7.31 (5H, m, Ar*H*), 5.25 (2H, dd, *J* = 16.9 and 12.1 Hz, OCH_2Ph), 4.66 (1H, d, *J* = 7.9 Hz, C*H*OSi), 3.79 (3H, d, *J* = 5.0 and 4.9 Hz, OC$^{A}H_3$), 3.74 (3H, d, *J* = 5.0 Hz, OC$^{B}H_3$), 0.90 (9H, s, C(CH_3)$_3$), 0.08 (6H, s, Si(CH_3)$_2$); NMR (75 MHz, $CDCl_3$) δ_C = 168.4, 135.3, 128.7, 128.6, 128.6, 70.8 (d, *J* = 163), 67.6, 54.2 (d, *J* = 6.9 Hz), 54.0 (d, *J* = 6.8 Hz), 25.5, 18.4, -5.4 (d, *J* = 12.5 Hz); ^{31}P (122 MHz, $CDCl_3$) δ_P = 18.5; IR (thin film) υ_{max} (cm^{-1}): 1753 (C=O); HRMS (ESI): *m/z* 389.1576, $C_{12}H_{28}O_6PSi$ [M+H]$^+$ requires 389.1549.

(*S*)-Benzyl 2-((*tert*-butyldimethylsilyl)oxy)-3-(2,2-dimethyl-1,3-dioxolan-4-yl)acrylate 499



Preparation from **228** and **181** employing the same method as that used for **199** gave the title compound as a colourless oil (0.39 g, 85% yield). Major isomer: $[\alpha]_D^{24}$ 11.4 (*c* 2.01, $CDCl_3$); ^{1}H NMR (300 MHz, $CDCl_3$) δ_H = 7.37-7.28 (5H, m, Ph), 5.55 (1H, d, *J* = 7.4 Hz, C*H*=C), 5.30 (1H, q, *J* = 7.4 Hz, C*H*CH=C), 5.19 (2H, s, CH_2Ph), 4.20 (1H, dd, *J* = 8.1 and 6.8 Hz, C$H^{A}H^{B}$CH), 3.55 (1H, dd, *J* = 7.9 and 6.1 Hz, CH$^{A}H^{B}$CH), 1.32 (3H, s, CC$^{A}H_3$), 1.28 (3H, s, CC$^{B}H_3$), 0.89 (9H, s, C(CH_3)$_3$), 0.09 (6H, s, Si(CH_3)$_2$); ^{13}C NMR (75 MHz, $CDCl_3$) δ_C = 166.2, 142.0, 136.0, 128.9, 128.5, 128.3, 123.6, 109.3, 72.5, 74.5, 70.0, 26.7, 25.6, 18.2, -5.8, -6.0. IR. (thin film) υ_{max} (cm^{-1}): 1726 (m, C=O); HRMS (ESI): *m/z* 414.1900, $C_{21}H_{32}O_5SiNa$ [M+Na]$^+$ requires 414.1917.

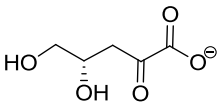
(*S*)-Benzyl 3-(2,2-dimethyl-1,3-dioxolan-4-yl)-2-oxopropanoate 229



Preparation from **499** employing the same method as that used for **200** afforded the title compound as a colourless oil (0.20 g, 79% yield). $[\alpha]_D^{20}$ +11.4 (*c* 0.90, $CDCl_3$); ^{1}H NMR

(300 MHz, $CDCl_3$) δ_H =7.67-7.28 (5H, m, Ph), 5.29 (2H, s, C*H*$_2$Ph), 5.52 (1H, quintet, *J* = 6.3 Hz, CH_2CHCH_2), 4.18 (1H, dd, *J* = 7.9 and 5.4 Hz, $CH^AH^BOC(CH_3)_2$), 3.59 (1H, dd, *J* = 8.5 and 6.3 Hz, $CH^AH^BOC(CH_3)_2$), 3.30 (1H, 1H, dd, *J* = 17.6 and 6.3 Hz, $CH^AH^BC{=}O$), 3.00 (1H, dd, *J* = 17.6 and 6.7 Hz, $CH^AH^BC{=}O$), 1.36 (3H, s, CC^AH_3), 1.30 (3H, s, CC^BH_3); ^{13}C NMR (75 MHz, $CDCl_3$) δ_C = 191.7, 160.3, 134.4, 129.0, 128.8, 128.7, 109.4, 71.2, 69.2, 68.3, 44.0, 26.9, 25.5; IR (thin film) u_{max} (cm^{-1}): 1726 (m, C=O); HRMS (ESI): *m/z* 301.1076, $C_{15}H_{18}NaO_5$ $[M+Na]^+$ requires 301.1052.

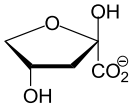
(*S*)-4,5-Dihydroxy-2-oxopentanoic acid D-KDX



Method A: D_2O (2 mL) was added to a toluene-d_8 (0.1 mL) solution of **223** (0.02 g, 0.092 mmol), followed by CalB (0.01 g) and the reaction was stirred at 40 °C and monitored by ^{1}H NMR. After 6 hours the reaction was complete and the reaction mixture was concentrated *in vacuo* to afford the residue as a yellow oil (0.013 g, 95% yield).

Method B: Water (5 mL) was added to a toluene (0.25 mL) solution of **223** (0.2 g, 0.92 mmol) and the mixture was carefully acidifed to pH 1.9 by the dropwise addition of 1 M $HCl_{(aq)}$. After stirring at room temperature for 24 hours, sufficient 1 M $NaOH_{(aq)}$ was added to neutralise the mixture. Novozym 435 (0.2 g) was added and the reaction mixture was heated to 40 °C, with the pH mantained at 6-7.5 by the careful addition of 1 M $NaOH_{(aq)}$. After 5 hours the mixture was filtered and concentrated *in vacuo* to give a yellow oil (0.128 g, 94% crude yield). This product could be further purified by silica gel chromatography (CH_2Cl_2-MeOH-H_2O, 5:5:1) to furnish the title compound as a yellow oil (0.065 g, 48% yield).

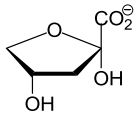
α-Furanose isomer (33%)



^{1}H NMR (500 MHz, D_2O) δ_H = 4.55-4.49 (1H, m, C*H*$OHCH_2$), 4.05 (1H, dd, *J* = 9.8 and 4.7 Hz, CH^AH^BCH), 3.95 (1H, dd, *J* = 9.8 and 2.5 Hz, CH^AH^BCH), 2.40 (1H, dd, *J* = 14.3 and

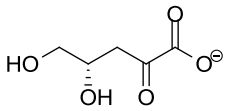
6.3 Hz, C*H*^{A}H^{B}C=O), 1.98 (1H, m, CH^{A}*H*^{B}C=O); ^{13}C NMR (126 MHz, D_2O) δ_C = 177.0, 103.8, 75.7, 71.4, 43.9.

β-Furanose isomer (37%)



^{1}H NMR (500 MHz, D_2O) δ_H = 4.51-4.47 (1H, m, C*H*OHCH$_2$), 4.09 (1H, dd, *J* = 9.7 and 4.3 Hz, C*H*^{A}H^{B}CH), 3.84 (1H, dd, *J* = 9.7 and 2.1 Hz, CH^{A}*H*^{B}CH), 2.26-2.21 (2H, m, C*H*$_2$C=O); ^{13}C NMR (126 MHz, D_2O) δ_C = 176.9, 103.9, 75.0, 70.6, 44.2.

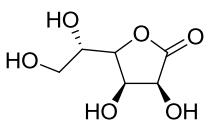
Acyclic isomer (30%)



^{1}H NMR (500 MHz, D_2O) δ_H = 4.18-4.11 (1H, m, C*H*OHCH$_2$), 3.55 (1H, dd, *J* = 11.8 and 4.2 Hz, C*H*^{A}H^{B}CH), 3.48 (1H, dd, *J* = 11.8 and 6.4 Hz, CH^{A}*H*^{B}CH), 2.92 (1H, dd, *J* = 17.1 and 4.4 Hz, C*H*^{A}H^{B}C=O), 2.84 (1H, dd, *J* = 17.1 and 8.3 Hz, CH^{A}*H*^{B}C=O); ^{13}C NMR (126 MHz, D_2O) δ_C = 203.8, 169.5, 67.5, 65.0, 42.8.

Mixture of 3 isomers: $[\alpha]_D^{26}$ +13.5 (*c* 4.75, H_2O-MeOH, 1:1); IR (thin film) υ_{max} (cm^{-1}): 3359 (br., OH); 2476 (br., CO_2H), 1756 (m, C=O), 1710 (m, C=O), 1602 (s, C=O); HRMS (ESI): *m/z* 147.0328, $C_5H_7O_5$ [M-H]$^{-}$ requires 147.0299.

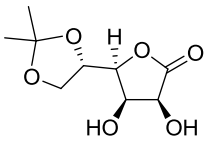
(3*S*,4*R*,5*R*)-5-((*S*)-1,2-Dihydroxyethyl)-3,4,5-trihydroxydihydrofuran-2(3H)-one[21] 231



L-Ascorbic acid (9.50 g, 54 mmol) was dissolved in water (70 mL) and charged into a Parr autoclave. After flushing with N_2, 10% Pd/C (0.9 g) was added and the reaction heated at 50 °C under a H_2 pressure of 130 psi. After 72 hours the reaction mixture was filtered through celite and the filtrate concentrated to a white solid. This was further purified by

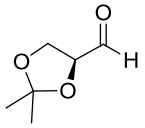
recrystallisation (EtOAc/MeOH) to give the title compound as white crystalline needles (8.00 g, 83% yield). $[\alpha]_D^{20}$ +54.0 (*c* 2.0, H_2O) (lit.[22] $[\alpha]_D^{23}$ +54.8 (*c* 4.0, H_2O)); ^{1}H NMR (500 MHz, DMSO-d_6) δ_H = 5.81 (1H, d, *J* = 7.5 Hz, CHO*H*C=O), 5.34 (1H, d, *J* = 7.6 Hz, CHO*H*CHOH), 4.98 (1H, d, *J* = 5.3 Hz, CHO*H*CH_2OH), 4.66 (1H, t, *J* = 5.7 Hz, CH_2O*H*), 4.44 (1H, dd, *J* = 7.6 and 4.6 Hz, C*H*OHC=O), 4.23 (1H, dd, *J* = 8.3 and 2.8 Hz, C*H*OC=O), 4.18 (1H, dd, *J* = 7.2 and 4.0 Hz, C*H*OHCHOH), 3.77-3.72 (1H, m, C*H*OHCH_2OH), 3.55-3.50 (1H, m, C*H*^{A}H^{B}OH), 3.50-3.44 (1H, m, CH^A*H*^{B}OH); ^{13}C NMR (75 MHz, DMSO-d_6) δ_C = 176.6, 81.2, 71.1, 70.4, 69.8, 62.3; IR (thin film) u_{max} (cm^{-1}): 3547 (m, OH), 3467 (m, OH), 3214 (br., OH), 1779 (s, C=O); HRMS (ESI): *m/z* 201.0388, $C_6H_{10}NaO_6$ [M+Na]$^+$ requires 201.0375.

(3a*S*,6*S*,6a*S*)-6-((*R*)-1,2-Dihydroxyethyl)-2,2-dimethyldihydrofuro[3,4-d][1,3]dioxol-4(3aH)-one[23] 233



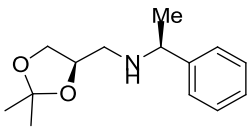
A DMF (33 mL) solution of **231** (3.67 g, 20.6 mmol) was cooled to 10 °C. *para*-toluenesulfonic acid monohydrate (0.03 g, 0.16 mmol) was added, followed by dropwise addition of isoprenyl methyl ether (2.57 ml, 26.8 mmol) and the solution was stirred at room temperature for 24 hours. It was then treated with Na_2CO_3.10H_2O (7.00 g, 24 mmol) and stirred vigirously for a further 2 hours. After filtering through celite the filtrates were concentrated *in vacuo* to a yellow oil, which crystallized upon addition of toluene. The title compound was removed by filtration, washed with petrol-ethanol (9:1) and dried *in vacuo* to a yellow crystalline solid (3.20 g, 72% yield). $[\alpha]_D^{25}$ +42.9 (*c* 1.4, DMSO) (lit.[23] $[\alpha]_D^{20}$ +38.3 (*c* 0.7, MeOH)); mp. 154-158 °C (lit.[23] mp. 167-168 °C); ^{1}H NMR (300 MHz, DMSO-d_6) δ_H = 5.82 (1H, br. s, O*H*), 5.39 (1H, br. s, O*H*), 4.34 (1H, d, *J* = 4.0 Hz, C*H*OHC=O), 4.24-4.08 (3H, m, C*H*OHC*H*OC*H*OH), 3.98 (1H, dd, *J* = 8.7 and 6.3 Hz, C*H*^{A}H^{B}COH), 3.67 (1H, dd, *J* = 8.3 and 6.1 Hz, CH^A*H*^{B}COH), 1.26(3H, s, CC^A*H*$_3$), 1.20 (3H, s, CC^B*H*$_3$); ^{13}C NMR (75 MHz, DMSO-d_6) δ_C = 176.3, 109.3, 81.6, 75.4, 70.5, 69.5, 64.7, 26.9, 25.6; IR (thin film) u_{max} (cm^{-1}): 3451 (br., OH), 1759 (s, C=O); HRMS (ESI): *m/z* 241.0707, $C_9H_{14}NaO_6$ [M+Na]$^+$ requires 241.0688.

2,3-*O*-isopropylidene-L-glyceraldehyde[23] 234



An aqueous (16 mL) suspension of **233** (3.47 g, 15.9 mmol) was cooled to 0 °C and the pH adjusted to 5.5 by the careful addition of 1 M $NaOH_{(aq)}$. $NaIO_4$ (6.8 g, 31.8 mmol) was added portion-wise over 5 minutes and a pH between 5–6 was maintained throughout the reaction. The reaction was stirred at room temperature and monitored by TLC (Et_2O-petrol, 2:1). After 2 hours **233** was fully consumed, so the solution was saturated with $NaCl_{(s)}$ and the resultant suspension filtered. The pH of the filtrates was adjusted to 7 before extraction with Et_2O (5 x 20 mL). The combined organic extracts were dried over anhydrous $MgSO_4$, filtered and concentrated at 55 °C under atmospheric pressure. Distillation of the residue yielded the title compound as a colourless oil (1.42 g, 69% yield). $[\alpha]_D^{20}$ -50 (*c* 0.5, $CDCl_3$) (lit.[24] $[\alpha]_D^{25}$ -54.9 (*c* 3.4, $CHCl_3$)); bpt. 67-72 °C @ 31 Torr (lit.[23] bpt. 64-66 °C @ 35 Torr); R_f = 0.24 (Et_2O-petrol, 2:1); ^{1}H NMR (300 MHz, $CDCl_3$) δ_H = 9.77 (1H, d, *J* = 2.02 Hz, C*H*O), 4.44 (1H, ddd, *J* = 7.3, 4.7 and 1.8 Hz, C*H*CHO), 4.22 (1H, dd, *J* = 8.8 and 7.4 Hz, CH^AH^B), 4.15 (1H, dd, *J* = 8.8 and 4.8 Hz, CH^AH^B), 1.54 (3H, s, C^AH_3), 1.47 (3H, s, C^BH_3); ^{13}C NMR (75 MHz, $CDCl_3$) δ_C = 201.9, 111.3, 79.9, 65.6, 26.3, 25.2; IR (thin film) υ_{max} (cm^{-1}): 1732 (m, C=O); HRMS (ESI): *m/z* 131.0713, $C_6H_{11}O_3$ $[M+H]^+$ requires 131.0708.

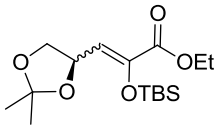
(*S*)-*N*-(((*R*)-2,2-Dimethyl-1,3-dioxolan-4-yl)methyl)-1-phenylethanamine 235



Preparation from **234** using the same method as that used for **187** afforded the title compound as a white solid (29 mg, 90% yield). $[\alpha]_D^{30}$ +46.0 (*c* 1.45, $CDCl_3$); ^{1}H NMR (300 MHz, $CDCl_3$) δ_H = 7.29-7.19 (5H, m, Ph), 4.16-4.07 (1H, m, C*H*CH_2NH), 3.91 (1H, dd, *J* = 8.0 and 6.3 Hz, CH^AH^{B}CH), 3.70 (1H, q, *J* =6.6 Hz, C*H*CH_3), 3.48 (1H, dd, *J* = 8.1 and 6.9 Hz, CH^AH^BCH), 2.55 (1H, dd, *J* = 11.9 and 7.4 Hz, CH^AH^{B}NH), 2.43 (1H, dd, *J* = 11.9 and

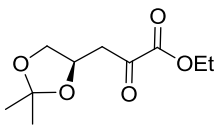
4.2 Hz, CH^ACH^BNH), 1.58 (1H, br. s, N*H*), 1.33 (3H, s, $CC^AH_3C^BH_3$), 1.32 (3H, d, *J* = 6.6 Hz, $CHCH_3$), 1.27 (3H, s, $CC^AH_3C^BH_3$); ^{13}C NMR (75 MHz, $CDCl_3$) δ_C = 145.5, 128.5, 127.0, 126.5, 109.1, 75.9, 67.7, 58.6, 50.8, 26.9, 25.5, 24.6; IR (thin film) u_{max} (cm^{-1}): 3027 (w, Csp^2-H), 2986 (w, Csp^3-H);; HRMS (ESI): *m/z* 258.1451, $C_{14}H_{21}NNaO_2$ $[M+Na]^+$ requires 258.1470.

(*R*)-Ethyl 2-(*tert*-butyldimethylsilyl)oxy)-3-(2,2-dimethyl-1,3-dioxolan-4-yl)acrylate 236



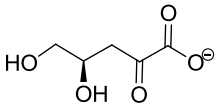
Aldehyde **234** was reacted with phosphonate ester **1** in the same way described for **199** to give the title compound as a colourless oil (0.50 g, 70% yield). Major isomer: $[\alpha]_D^{24}$ -12.9 (*c* 0.62, $CHCl_3$); ^1H NMR (300 MHz, $CDCl_3$) δ_H = 5.33 (1H, d, *J* = 7.7 Hz, C*H*=C), 5.17 (1H, app. q, *J* = 6.6 Hz, C*H*CH=C), 4.12 (1H, dd, *J* = 8.0 and 6.6 Hz, CH^AH^BCH), 4.07 (2H, q, *J* = 7.1 Hz, OCH_2), 3.41 (1H, dd, *J* = 8.2 and 6.9 Hz, CH^AH^BCH), 1.28 (3H, s, CC^AH_3), 1.22 (3H, s, CC^BH_3), 1.16 (3H, t, *J* = 7.2 Hz, CH_2CH_3), 0.79 (9H, s, $C(CH_3)_3$), 0.00 (6H, s, $Si(CH_3)_2$); ^{13}C NMR (75 MHz, $CDCl_3$) δ_C = 164.2, 142.0, 123.7, 109.3, 72.5, 70.0, 61.1, 26.7, 25.6, 18.2, 14.1, -4.8, -4.9. Minor isomer: ^1H NMR (300 MHz, $CDCl_3$) δ_H = 5.81 (1H, d, *J* = 8.3 Hz, C*H*=C), 4.92-4.82 (1H, m, C*H*CH=C), 4.06 (2H, q, *J* = 7.0 Hz, OCH_2), 3.97 (1H, dd, *J* = 8.0 and 6.2 Hz, CH^AH^BCH) 3.42 (1H, dd, *J* = 8.1 and 6.7 Hz, CH^AH^BCH), 1.29 (3H, s, CC^AH_3), 1.24 (3H, s, CC^BH_3), 1.15 (3H, t, *J* = 7.2 Hz, CH_2CH_3), 0.80 (9H, s, $C(CH_3)_3$), 0.03 (6H, s, $Si(CH_3)_2$). IR (thin film) u_{max} (cm^{-1}): 1718 (m, C=O); HRMS (ESI): *m/z* 353.1780, $C_{16}H_{30}NaO_5Si$ $[M+Na]^+$ requires 353.1760.

(*R*)-Ethyl 3-(2,2-dimethyl-1,3-dioxolan-4-yl)-2-oxopropanoate 237



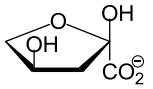
Compound **236** was deprotected in the same way described for **200** to give the title compound as a yellow oil (0.28 g, 86% yield). $[\alpha]_D^{25}$ -8.9 (*c* 0.45, CH_2Cl_2); ^{1}H NMR (300 MHz, $CDCl_3$) δ_H = 4.47 (1H, qt, *J* = 6.3 Hz, CH_2CHCH_2), 4.27 (2H, q, *J* = 7.2 Hz, OCH_2CH_3), 4.14 (1H, dd, *J* = 8.4 and 6.0 Hz, $CH^AH^BOC(CH_3)_2$), 3.54 (1H, *J* = 8.5 and 6.3 Hz, $CH^AH^BOC(CH_3)_2$), 3.24 (1H, dd, *J* = 17.7 and 6.2 Hz, $CH^AH^BC{=}O$), 2.95 (1H, dd, *J* = 17.7 and 6.8 Hz, $CH^AH^BC{=}O$), 1.35 (3H, s, CC^AH_3 C^BH_3), 1.31 (3H, t, *J* = 7.1 Hz, OCH_2CH_3), 1.29, (3H, s, C $C^{BA}H_3C^BH_3$); ^{13}C NMR (75 MHz, $CDCl_3$) δ_C = 192.0, 160.5, 109.3, 71.2, 69.1, 62.7, 43.8, 26.8, 25.5, 14.0; IR (thin film) u_{max} (cm^{-1}): 1726 (m, C=O); HRMS (ESI): *m/z* 239.0886, $C_{10}H_{16}$ Na O_5 $[M+Na]^+$ requires 239.0895.

(*R*)-4,5-Dihydroxy-2-oxopentanoic acid L-KDA



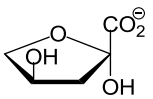
Compound **237** was deprotected and hydrolysed using method 2 developed for the synthesis of **D-KDX** to give the title compound as a yellow oil (0.08 g, 87% yield).

α-Furanose isomer (33%)



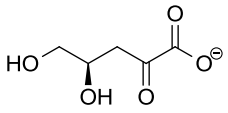
^{1}H NMR (500 MHz, $CDCl_3$) δ_H = 4.52-4.48 (1H, m, $CHOHCH_2$), 4.04 (1H, dd, *J* = 9.8 and 4.7 Hz, CH^AH^BCH), 3.93 (1H, dd, *J* = 9.8 and 2.5 Hz, CH^AH^BCH), 2.39 (1H, dd, *J* = 14.3 and 6.2 Hz, CH^AH^BCOH), 1.97 (1H, app.d, *J* = 14.6 Hz, CH^AH^BCOH); ^{13}C NMR (126 MHz, $CDCl_3$) δ_C = 177.0, 103.8, 75.6, 71.3, 43.9.

β-Furanose isomer (37%)



^{1}H NMR (500 MHz, $CDCl_3$) δ_H = 4.50-4.46 (1H, m, $CHOHCH_2$), 4.07 (1H, dd, *J* = 9.7 and 4.3 Hz, CH^AH^BCH), 3.83 (1H, dd, *J* = 9.7 and 2.1 Hz, CH^AH^BCH), 2.25-2.19 (2H, m, CH_2COH); ^{13}C NMR (126 MHz, $CDCl_3$) δ_C = 176.9, 103.8, 75.0, 70.6, 44.2.

Acyclic isomer (30%)



^{1}H NMR (500 MHz, $CDCl_3$) δ_H = 4.15-4.10 (1H, m, C*H*OHCH$_2$), 3.54 (1H, dd, *J* = 11.8 and 4.2 Hz, C*H*^{A}H^{B}CH), 3.46 (1H, dd, *J* = 11.8 and 6.4 Hz, CH^A*H*^{B}CH), 2.90 (1H, dd, *J* = 17.1 and 4.3 Hz, C*H*^{A}H^{B}C=O), 2.83 (1H, dd, *J* = 17.1 and 8.4 Hz, CH^A*H*^{B}C=O); ^{13}C NMR (126 MHz, $CDCl_3$) δ_C = 203.8, 169.5, 67.5, 65.0, 42.8;

Equilibrium mixture of 3 isomers: $[\alpha]_D^{25}$ -13.3 (*c* 4.95, H_2O-MeOH, 1:1); IR (thin film) υ_{max} (cm^{-1}): 3302 (br., OH); 2496 (br., CO_2H), 1752 (m, C=O), 1710 (m, C=O), 1603 (s, C=O); HRMS (ESI): *m/z* 147.0212, $C_5H_7O_5$ [M-H]$^-$ requires 147.0299.

Expression and Purification of KDG aldolase

KDG aldolase was expressed using the pET-3a expression vector (Novagen, Nottingham, UK) with the gene cloned into the *Nde*I and *Bam*HI restriction sites. One-litre cultures of *E. coli* BL21(DE3) (Novagen) containing the vector were grown overnight at 37 °C without induction. Cells were collected by centrifugation and re-suspended at 0.2 g/mL in water. These cells were lysed by two passes through a cell disruptor (one-shot model, Constant Systems, Warwick, UK) at 200MPa before heat precipitation at 95 °C for 30 min. Debris was removed by centrifugation at 18,000 x *g* for 30 min, and the resulting KDG-aldolase sample lyophilized. The crude residue was first purified by gel filtration on a Sephacryl S-300 HR column (5cm x 44cm) in 20 mM Tris/HCl buffer (pH 8.5). Gel-filtration fractions showing KDG-aldolase activity were then applied to a HiTrap Q anion-exchange column (previously equilibriated in 20 mM Tris/HCl buffer (pH 8.5)) and eluted with 20 mM Tris/HCl buffer (pH 8.5) followed by a gradient of 0-2 M NaCl in 20 mM Tris/HCl buffer (pH 8.5). The fractions containing pure KDG aldolase by SDS/PAGE were lyophilised to afford a concentrated aqueous solution of KDG aldolase. The concentration of enzyme was determined to be 2.07 g/ml using a NanoVue spectrophotometer.

Kinetic Study Using Modified Thiobarbituric Acid Assay[3][325]

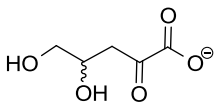
Different concentrations of **D-KDX** (or **L-KDA**) (2.5-80 mM) in 50 mM sodium phosphate buffer (pH 6.0) were prepared with a total volume of 100 µl. These samples were then incubated with purified KDG aldolase (5 µl, 2.07g/mL) at 50 °C for 10 minutes before each

kinetic assay was terminated by the addition of trichloroacetic acid (10 µl, 12% w/v). Precipitated proteins were removed by centrifugation (9,000 x *g* for 5 minutes) and 50 µl of the supernatant oxidized by addition of periodic acid (125 µl, 25 mM in 0.125 M H_2SO_4 solution) followed by incubation at room temperature for 20 minutes. This oxidation step was terminated by the addition of sodium arsenite (250 µl, 2% w/v in 0.5 M HCl). Thiobarbituric acid(aq) **249** (1 mL, 0.3% w/v) was then added and the chromophore developed by heating at 100 °C for 10 min. A 25 µl sample of this solution was then removed, diluted with 1400 µl of water, and the absorbance of the solution determined at 549 nm. The absorption coefficient of the resultant chromophore was determined to be 14519 $M^{-1}cm^{-1}$.

Preparation of bakers yeast expressing pyruvate decarboxylase[44]

Type II bakers yeast (7.0 g) was suspended in cold water (100 mL) and stirred for 18 hours at <4 °C. The cells were harvested by centrifugation (18,000 x *g* for 30 minutes), washed twice with cold water (2x100 mL, <4 °C), before resuspension of the harvested cells in cold water (20 mL) and storage at <4 °C for future use in the KDG aldolase catalysed reaction of glycolaldehyde **14** and pyruvate **7**.

4,5-Dihydroxy-2-oxopentanoate 251

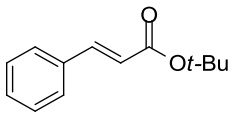


Glycolaldehyde **14** (0.270 g, 4.54 mmol) and sodium pyruvate **4** (0.800 g, 9.08mmol) were added to a solution of KDG aldolase (5 µl) in 20 mM Tris/HCl buffer (23 mL, pH 6.5). The reaction was heated at 50 °C using a shaking incubator, with reaction progress monitored in-process by analysis using an Agilent 1200 HPLC fitted with a Bio-Rad Aminex HPX-87H column (300mm x 7.8mm) (0.6 mL/min, 1 M formic acid, 60 °C), using a refractive index detector for peak detection. Once all the glycolaldehyde **14** had been consumed, the reaction mixture was quenched by acidification to pH 2 with 1 M HCl(aq) and then neutralised to pH 7.0 after 2 hours using 2 M NaOH(aq). An air bubbler was attached to the reaction vessel and a suspension of yeast cells (20 mL) expressing pyruvate decarboxylase added. The resultant suspension was then stirred for 3 hours, with the pH

maintained above 6.5 *via* careful addition of 1 M $NaOH_{(aq)}$, until HPLC analysis revealed that all of the excess pyruvate had been consumed. The residual yeast cells were removed by centrifugation (18,000 x *g* for 3 x 30 minutes) and the mother liquors concentrated to afford a crude reaction product that was purified by flash chromatography on silica gel (CH_2Cl_2-MeOH-H_2O, 5:5:1) to afford the title compound as a yellow oil (0.363 g, 54%). $[\alpha]_D^{26}$ +0.7 (*c* 4.95, H_2O-MeOH, 1:1) with spectroscopic data matching **D-KDX** and **L-KDA**.

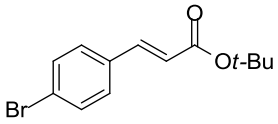
6.4 Procedures and Data for Chapter 4

(*E*)-*tert*-Butyl 3-phenylacrylate[26] 305



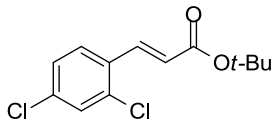
To a solution of iodobenzene **337** (2.69 g, 13.2 mmol) in MeCN (80 mL) under an inert atmosphere were added successively DIPEA (6.90 ml, 39.6 mmol), *tert*-butyl acrylate **338** (2.12 ml, 14.5 mL), tri(*o*-tolyl)phosphine (0.40g, 13.2 mmol) and palladium(II) acetate (0.15 g, 0.66 mmol). The mixture was heated at reflux for 17 hours and monitored by TLC (EtOAc-petrol, 1:30). The suspension was then cooled to room temperature, diluted with water (60 mL) and extracted with EtOAc (3 x 50 ml3). The combined organic extracts were washed with brine and water, dried over anhydrous $MgSO_4$ and concentrated *in vacuo* to a purple oil. The residue was purified by flash chromatography (EtOAc-petrol, 1:30) to afford the title compound as a yellow oil (1.94 g, 72% yield). R_f = 0.39 (EtOAc-petrol, 1:30); ^{1}H NMR (300 MHz, $CDCl_3$) δ_H = 7.51 (1H, d , *J* = 16.0 Hz, C*H*=CHCO$_2$), 7.47-7.25 (5H, m, Ph), 6.30 (1H, d, *J* = 16.0 Hz, CH=C*H*CO$_2$), 1.46 (9H, s, C(C*H*$_3$)$_3$); ^{13}C NMR (75 MHz, $CDCl_3$) δ_C = 166.3, 143.6, 134.7, 123.0, 128.8, 128.0, 120.2, 80.5, 28.2; IR (thin film) υ_{max} (cm^{-1}): 1703 (s, C=O); HRMS (ESI): *m/z* 227.1025, $C_{13}H_{16}NaO_2$ $[M+Na]^+$ requires 227.1043.

(*E*)-*tert*-Butyl 3-(4-bromophenyl)acrylate[27] 336b



To a solution of *tert*-butyl 2-(diethoxyphosphoryl)acetate **335** (0.50 g, 1.98 mmol) in dry THF (25 mL) at room temperature under an inert atmosphere, was added methylmagnesium bromide (0.66 ml, 1.98 mmol) and the mixture stirred for 15 minutes. A dry THF (12.5 mL) solution of 4-bromobenzaldehyde was then added dropwise and the reaction mixture heated at reflux for 18 hours. After cooling to room temperature, saturated $NH_4Cl_{(aq)}$ was added and the mixture was extracted with Et_2O (3 x 30 mL). The combined organic extracts were washed with brine and water, dried over anhydrous $MgSO_4$, filtered and concentrated *in vacuo*. The residue was purified by silica gel chromatography (EtOAc-petrol, 1:30) affording the title compound as a white solid (0.404 g, 72% yield). Mp. 53-55 °C (lit.[27] mpt. 52-53 °C); ^{1}H NMR (300 MHz, $CDCl_3$) δ_H = 7.47 (1H, d, *J* = 15.8 Hz, C*H*=CHCO$_2$), 7.42 (2H, d, *J* = 6.8 Hz, BrCCHC*H*), 7.29 (2H, d, *J* = 6.8 Hz, BrCC*H*CH), 6.31 (1H, d, *J* = 15.8 Hz, CH=C*H*CO$_2$), 1.46 (9H, s, C(C*H*$_3$)$_3$); ^{13}C NMR (75 MHz, $CDCl_3$) δ_C = 166.1, 142.2, 133.6, 132.1, 129.36, 124.2, 120.9, 80.8, 28.2; IR (thin film) υ_{max} (cm^{-1}): 1704 (s, C=O); HRMS (ESI): *m/z* 305.0129, $C_{13}H_{15}BrNaO_2$ $[M+Na]^+$ requires 305.0153.

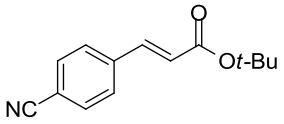
(*E*)-*tert*-Butyl 3-(2,4-dichlorophenyl)acrylate 336c



Preparation from 2,4-dichlorobenzaldehyde employing the same method as that used for **336b** afforded the title compound as a colourless oil (0.59 g, 78% yield). R_f = 0.35 (EtOAc-petrol, 1:50); ^{1}H NMR (300 MHz, $CDCl_3$) δ_H = 7.92 (1H, d, *J* = 16.0 Hz, C*H*=CHCO$_2$), 7.54 (1H, d, *J* = 8.5 Hz, C*H*CCl), 7.43 (1H, d, *J* = 2.0 Hz, CClC*H*CCl), 7.25 (1H, dd, *J* = 8.6 and 2.0 Hz, CC*H*CH), 6.35 (1H, d, *J* = 16.1 Hz, CH=C*H*CO$_2$), 1.54 (9H, s, C(C*H*$_3$)$_3$); ^{13}C NMR (75 MHz, $CDCl_3$) δ_C = 165.6, 138.1, 136.0, 135.4, 131.5, 129.9, 128.3,

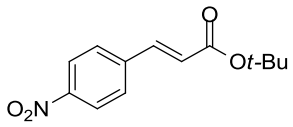
127.5, 123.2, 81.0, 28.2; IR (thin film) υ_{max} (cm^{-1}): 1706 (s, C=O); HRMS (ESI): *m/z* 295.0244, $C_{13}H_{14}Cl_2NaO_2$ $[M+Na]^+$ requires 295.0269.

(*E*)-*tert*-Butyl 3-(4-cyanophenyl)acrylate[27] 336d



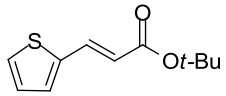
Preparation from 4-formylbenzonitrile employing the same method as that used for **336b** afforded the title compound as a white solid (0.59 g, 82% yield). Mp. 140-142 °C (lit.[27] mp. 136-138 °C); R_f = 0.49 (EtOAc-petrol, 1:10); ^{1}H NMR (300 MHz, $CDCl_3$) δ_H = 7.67 (2H, d, *J* = 7.6 Hz, NCCC*H*), 7.58 (2H, d, *J* = 7.6 Hz, NCCCHC*H*), 7.55 (1H, d, *J* = 16.1 Hz, C*H*=CHCO$_2$), 6.45 (1H, d, *J* = 16.1 Hz, CH=C*H*CO$_2$), 1.54 (9H, s, C(C*H*$_3$)$_3$); ^{13}C NMR (75 MHz, $CDCl_3$) δ_C = 165.4, 141.1, 139.0, 132.6, 128.3, 123.8, 118.4, 113.1, 81.2, 28.1; IR (thin film) υ_{max} (cm^{-1}): 2226 (m, CN), 1702 (s, C=O); HRMS (ESI): *m/z* 252.0990, $C_{14}H_{15}NNaO_2$ $[M+Na]^+$ requires 252.1000.

(*E*)-*tert*-Butyl 3-(4-nitrophenyl)acrylate 336e



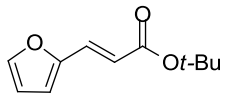
Preparation from 4-nitrobenzaldehyde employing the same method as that used for **336b** afforded the title compound as a colourless oil (0.35 g, 69% yield). R_f 0.43 (EtOAc-petrol, 1:15); ^{1}H NMR (300 MHz, $CDCl_3$) δ_H = 8.14 (2H, d, *J* = 8.7 Hz, O$_2$NCC*H*), 7.57 (2H, d, *J* = 8.7 Hz, O$_2$NCCHC*H*), 7.53 (1H, d, *J* = 16.0 Hz, C*H*=CHCO$_2$), 6.42 (1H, d, *J* = 15.9 Hz, CH=C*H*CO$_2$), 1.46 (9H, s, C(C*H*$_3$)$_3$); ^{13}C NMR (75 MHz, $CDCl_3$) δ_C = 165.3, 148.3, 140.9, 140.6, 128.5, 124.5, 124.1, 81.5, 28.1; IR (thin film) υ_{max} (cm^{-1}): 1708 (s, C=O); HRMS (ESI): *m/z* 272.0878, $C_{13}H_{15}NNaO_4$ $[M+Na]^+$ requires 272.0899.

(*E*)-*tert*-Butyl 3-(thiophen-2-yl)acrylate 336f



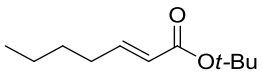
Preparation from thiophene-2-carbaldehyde employing the same method as that used for **336b** afforded the title compound as a colourless oil (0.60 g, 67% yield). R_f 0.41 (EtOAc-petrol, 1:50); ^{1}H NMR (300 MHz, $CDCl_3$) δ_H = 7.67 (1H, d, *J* = 15.7 Hz, C*H*=CHCO$_2$), 7.32 (1H, d, *J* = 5.1 Hz, C*H*S), 7.20 (1H, d, *J* = 3.5 Hz, C*H*CS), 7.02 (1H, dd, *J* = 5.1 and 3.6 Hz, CHC*H*CHS), 6.16 (1H, d, *J* = 15.7 Hz, CH=C*H*CO$_2$), 1.51 (9H, s, C(C*H*$_3$)$_3$); ^{13}C NMR (75 MHz, $CDCl_3$) δ_C = 166.2, 139.8, 136.1, 130.5, 128.0, 119.0, 80.5, 28.2; IR (thin film) υ_{max} (cm^{-1}): 1699 (s, C=O); HRMS (ESI): *m/z* 233.0591, $C_{11}H_{14}NaO_2S$ [M+Na]$^+$ requires 233.0612.

(*E*)-*tert*-Butyl 3-(furan-2-yl)acrylate 336g



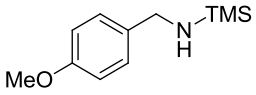
Preparation from furan-2-carbaldehyde employing the same method as that used for **336b** afforded the title compound as a yellow oil (0.51 g, 65% yield). R_f 0.13 (EtOAc-petrol, 1:50); ^{1}H NMR (300 MHz, $CDCl_3$) δ_H = 7.38 (1H, d, *J* = 1.6 Hz, C*H*O), 7.26 (1H, d, *J* = 15.9 Hz, C*H*=CHCO$_2$), 6.50 (1H, d, *J* = 3.4 Hz, C*H*CO), 6.38 (1H, dd, *J* = 3.4 and 1.8 Hz, CHC*H*CHO), 6.18 (1H, d, *J* = 15.9 Hz, CH=C*H*CO$_2$), 1.44 (9H, s, C(C*H*$_3$)$_3$); ^{13}C NMR (75 MHz, $CDCl_3$) δ_C = 166.4, 151.2, 144.4, 130.1, 118.0, 114.0, 112.1, 80.4, 28.2; IR (thin film) υ_{max} (cm^{-1}): 1701 (s, C=O); HRMS (ESI): *m/z* 217.0875, $C_{11}H_{14}NaO_3$ [M+Na]$^+$ requires 217.0841.

(*E*)-*tert*-Butyl hept-2-enoate 341



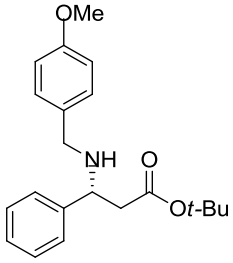
To a solution of hex-1-ene **339** (2.89 ml, 13.6 mmol) in CH_2Cl_2 (29 mL) under inert atmosphere was added *tert*-butyl acrylate **340** (5.92 ml, 40.8 mmol) followed by Grubbs 2nd generation catalyst (0.10 g). The reaction mixture was stirred at room temperature for 24 hours and then filtered through celite and concentrated *in vacuo*. Distillation of the residue furnished the title compound as a colourless oil (1.42 g, 55% yield). Bp. 90 °C @ 0.8 Torr; ^{1}H NMR (250 MHz, $CDCl_3$) δ_H = 6.78 (1H, dt, *J* = 15.7 and 6.9 Hz, CH_2CH=CH), 5.66 (1H, dt, *J* = 15.7 and 1.5 Hz, CH_2CH=C*H*), 2.10 (2H, qd, *J* = 7.1 and 1.5 Hz, CH_2-CH_2-CH), 1.49 (9H, s, C(CH_3)$_3$), 1.37-1.18 (4H, m, $CH_3CH_2CH_2$), 0.93 (3H, t, *J* = 7.1 Hz, CH_3CH_2); ^{13}C NMR (75 MHz, $CDCl_3$) δ_C = 166.6, 148.5, 123.3, 80.3, 32.2, 30.6, 28.5, 22.6, 14.2; IR (thin film) υ_{max} (cm^{-1}): 1706 (s, C=O); HRMS (ESI): *m/z* 207.1343, $C_{11}H_{20}NaO_2$ $[M+Na]^+$ requires 207.1361.

***N*-(4-Methoxybenzyl)-1,1,1-trimethylsilanamine 325**



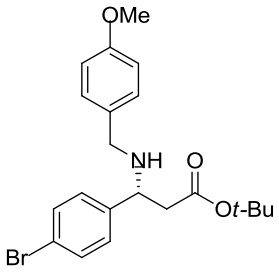
The reaction was conducted under inert atmosphere in oven dried glassware. To a stirred solution of *para*-methoxy-benzylamine (5.00 g, 36.4 mmol) in THF (140 mL) cooled to 0 °C was added *n*-butyllithium solution (2.3 M in hexanes, 6.6 ml, 38.2 mmol) dropwise. The reaction was stirred at room temperature for 8 hours and then cooled to 0 °C prior to the dropwise addition of chlorotrimethylsilane (4.67 ml, 36.8 mmol) in THF (40 mL). The reaction was heated at reflux for 12 hours, concentrated, triturated with pentane (2 x 100 mL) and concentrated *in vacuo* to afford the title compound as a yellow oil (5.59 g, 73% yield). ^{1}H NMR (300 MHz, $CDCl_3$) δ_H = 7.16 (2H, d, *J* = 8.6 Hz, $CH_3OCCHCH$), 6.82 (2H, d, *J* = 8.6 Hz, CH_3OCCH), 3.77 (2H, d, *J* = 7.8 Hz, CH_2N), 3.7 (3H, s, OCH_3), 1.90 (1H, br. s, N*H*), 0.00 (9H, s, Si(CH_3)$_3$); ^{13}C NMR (75 MHz, $CDCl_3$) δ_C = 158.2, 136.5, 129.1, 113.85, 55.2, 45.3, 0.0; IR (thin film) υ_{max} (cm^{-1}): 3305 (w, NH), 1505, 1499 (m, m, C=C).

(*R*)-*tert*-Butyl 3-((4-methoxybenzyl)amino)-3-phenylpropanoate 326a



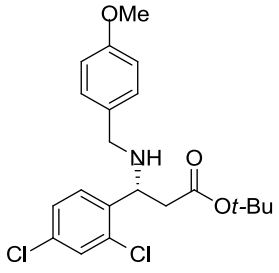
To a solution of amine **325** (1.81 g, 8.66 mmol) in dry toluene (23.1 mL) at -78 °C, under an inert atmosphere, was added *n*-butyllithium (2.5 M in hexanes, 3.46 ml, 8.66 mmol) dropwise. After stirring the mixture at -78 °C for 30 minutes, a dry toluene (11.4 mL) solution of chiral ligand **303** (2.62 g, 10.4 mmol) was added dropwise. After stirring the mixture for a further 30 minutes at -78 °C, a dry toluene (11.4 mL) solution of **305** (1.18 g, 5.78 mmol) and chlorotrimethylsilane (3.65 ml, 28.9 mmol) was added dropwise. The reaction mixture was stirred for 5 hours at -78 °C and then quenched by the careful addition of saturated $NH_4Cl_{(aq)}$. After allowing the suspension to stir-warm to room temperature saturated $NaHCO_{3(aq)}$ was added carefully and then extracted with EtOAC (3 x 100 mL). The combined organic extracts were washed with brine and water, dried over anhydrous $MgSO_4$, filtered and concentrated *in vacuo*. The residue was purified by silica gel chromatography (EtOAc-petrol, 1:5), affording the title compoundas a yellow oil (1.52 g, 77% yield). $[\alpha]_D^{26}$ +25.9 (*c* 3.2, CH_2Cl_2); R_f 0.18 (EtOAc-petrol, 1:5); ^{1}H NMR (300 MHz, $CDCl_3$) δ_H = 7.43-7.26 (5H, m, Ph), 7.22 (2H, d, *J* = 8.6 Hz, C*H*CHCOCH$_3$), 6.87 (2H, d, *J* = 8.6 Hz, CHC*H*COCH$_3$), 4.09 (1H, dd, *J* = 8.6 and 5.5 Hz, C*H*NH), 3.82 (3H, s, OC*H*$_3$), 3.61 (1H, d, *J* = 12.9 Hz, NHC*H*^{A}H^B), 3.51 (1H, d, *J* = 12.9 Hz, NHCH^A*H*^{B}), 2.68 (1H, dd, *J* = 15.3 and 8.7 Hz, C*H*^{A}CH^{B}CO$_2$), 2.56 (1H, dd, *J* = 15.3 and 5.6 Hz, CH^{A}C*H*^{B}CO$_2$), 2.20 (1H, br. s, N*H*), 1.40 (9H, s, C(C*H*$_3$)$_3$); ^{13}C NMR (75 MHz, $CDCl_3$) δ_C = 171.1, 158.6, 142.8, 132.6, 129.3, 128.5, 127.4, 113.8, 80.6, 59.1, 55.3, 50.8, 44.3, 28.0; IR (thin film) u_{max} (cm^{-1}): 1721 (s, C=O); HRMS (ESI): *m/z* 364.1911, $C_{21}H_{27}NNaO_3$ $[M+Na]^+$ requires 364.1889.

(*R*)-*tert*-Butyl 3-(4-bromophenyl)-3-((4-methoxybenzyl)amino)propanoate 326b



Preparation from **336b** employing the same method as that used for **326a** afforded the title compound as a white solid (0.64 g, 79% yield). $[\alpha]_D^{26}$ +38.6 (*c* 1.71, EtOAc); R_f 0.34 (EtOAc-petrol, 1:6); ^{1}H NMR (300 MHz, $CDCl_3$) δ_H = 7.40 (2H, d, *J* = 8.3 Hz, CHC*H*CBr), 7.18 (2H, d, *J* = 8.3 Hz, C*H*CHCBr), 7.09 (2H, d, *J* = 8.5 Hz, C*H*CHCOCH$_3$), 6.77 (2H, d, *J* = 8.5 Hz, CHC*H*COCH$_3$), 3.95 (1H, dd, *J* = 8.4 and 5.4 Hz, C*H*NH), 3.72 (3H, s, OC*H*$_3$), 3.48 (1H, d, *J* = 12.5 Hz, NHC*H*^{A}H^B), 3.38 (1H, d, *J* = 12.5 Hz, NHCH^A*H*^{B}), 2.52 (1H, dd, *J* = 15.4 and 8.4 Hz, C*H*^{A}CH^{B}CO$_2$), 2.40 (1H, dd, *J* = 15.4 and 5.4 Hz, CH^{A}C*H*^{B}CO$_2$), 1.94 (1H, br. s, N*H*), 1.30 (9H, s, C(C*H*$_3$)$_3$); ^{13}C NMR (75 MHz, $CDCl_3$) δ_C = 170.8, 158.6, 141.8, 132.2, 131.6, 129.3, 129.1, 121.1, 113.8, 80.9, 58.5, 55.3, 50.8, 44.1, 28.0; IR (thin film) υ_{max} (cm^{-1}): 1739 (s, C=O); HRMS (ESI): *m/z* 442.1006, $C_{21}H_{26}BrNNaO_3$ [M+Na]$^+$ requires 442.0994.

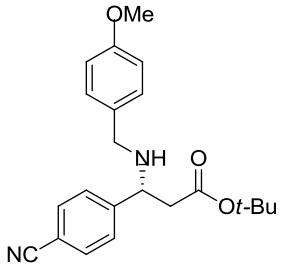
(*R*)-*tert*-Butyl 3-(2,4-dichlorophenyl)-3-((4-methoxybenzyl)amino)propanoate 326c



Preparation from **336c** employing the same method as that used for **326a** afforded the title compound as a white solid (0.52 g, 76% yield). $[\alpha]_D^{27}$ +24.1 (*c* 1.58, CH_2Cl_2); R_f 0.22 (EtOAc-petrol, 1:10); ^{1}H NMR (300 MHz, $CDCl_3$) δ_H = 7.48 (1H, d, *J* = 8.5 Hz, C*H*CHCCl), 7.29 (1H, d, *J* = 2.1 Hz, CClC*H*CCl), 7.19 (1H, dd, *J* = 8.4 and 2.1 Hz, CHC*H*CCl), 7.09

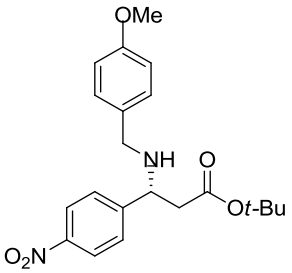
(2H, d, *J* = 8.6 Hz, C*H*CHCOCH$_3$), 6.75 (2H, d, *J* = 8.6 Hz, CHC*H*COCH$_3$), 4.44 (1H, dd, *J* = 8.6 and 4.5 Hz, C*H*NH), 3.70 (3H, s, OC*H*$_3$), 3.46 (1H, d, *J* = 12.5 Hz, NHC*H*^{A}H^B), 3.41 (1H, d, *J* = 12.8 Hz, NHCH^A*H*^{B}), 2.51 (1H, dd, *J* = 15.6 and 4.7 Hz, C*H*^{A}CH^{B}CO$_2$), 2.40 (1H, dd, *J* = 15.5 and 8.8 Hz, CH^{A}C*H*^{B}CO$_2$), 1.31 (9H, s, C(C*H*$_3$)$_3$); ^{13}C NMR (75 MHz, CDCl$_3$) δ_C = 170.6, 158.7, 138.5, 134.2, 133.3, 129.4, 129.3, 127.5, 113.8, 81.0, 55.3, 55.1, 51.0, 42.2, 28.1; IR (thin film) υ_{max} (cm^{-1}): 1723 (s, C=O); HRMS (ESI): *m/z* 432.1128, $C_{21}H_{25}Cl_2NNaO_3$ [M+Na]$^+$ requires 432.1110.

(*R*)-*tert*-Butyl 3-(4-cyanophenyl)-3-((4-methoxybenzyl)amino)propanoate 326d



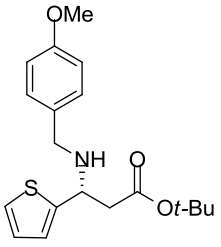
Preparation from **336d** employing the same method as that used for **326a** afforded the title compound as a yellow oil (0.23 g, 62% yield). $[\alpha]_D^{26}$ +42.7 (*c* 3.35, EtOAc)**;** R_f = 0.19 (EtOAc-petrol, 1:3); ^{1}H NMR (300 MHz, CDCl$_3$) δ_H = 7.56 (2H, d, *J* = 8.4 Hz, CHC*H*CCN), 7.42 (2H, d, *J* = 8.4 Hz, C*H*CHCCN), 7.07 (2H, d, *J* = 8.6 Hz, C*H*CHCOCH$_3$), 6.76 (2H, d, *J* = 8.6 Hz, CHC*H*COCH$_3$), 4.03 (1H, dd, *J* = 8.4 and 5.3 Hz, C*H*NH), 3.71 (3H, s, OC*H*$_3$), 3.46 (1H, d, *J* = 12.9 Hz, NHC*H*^{A}H^B), 3.38 (1H, d, *J* = 12.9 Hz, NHCH^A*H*^{B}), 2.53 (1H, dd, *J* = 15.4 and 8.3 Hz, C*H*^{A}CH^{B}CO$_2$), 2.41 (1H, dd, *J* = 15.4 and 5.4 Hz, CH^{A}C*H*^{B}CO$_2$), 2.09 (1H, br. s, N*H*), 1.29 (9H, s, C(C*H*$_3$)$_3$); ^{13}C NMR (75 MHz, CDCl$_3$) δ_C = 170.4, 158.7, 148.5, 132.4, 131.8, 129.3, 128.2, 118.9, 113.8, 111.2, 81.2, 58.8, 55.3, 50.9, 43.8, 28.0; IR (thin film) υ_{max} (cm^{-1}): 2226 (m, CN), 1703 (s, C=O); HRMS (ESI): *m/z* 389.1855, $C_{22}H_{26}N_2NaO_3$ [M+Na]$^+$ requires 389.1841.

(*R*)-*tert*-Butyl 3-((4-methoxybenzyl)amino)-3-(4-nitrophenyl)propanoate 326e



Preparation from **336e** employing the same method as that used for **326a** afforded the title compound as a yellow oil (0.33 g, 40% yield). $[\alpha]_D^{26}$ +35.7 (*c* 2.6, EtOAc); R_f 0.25 (EtOAc-petrol, 1:3); ^{1}H NMR (300 MHz, $CDCl_3$) δ_H = 8.12 (2H, d, *J* = 8.7 Hz, CHC*H*CNO_2), 7.47 (2H, d, *J* = 8.7 Hz, C*H*$CHCNO_2$), 7.06 (2H, d, *J* = 8.5 Hz, C*H*$CHCOCH_3$), 6.75 (2H, d, *J* = 8.5 Hz, CHC*H*$COCH_3$), 4.08 (1H, dd, *J* = 8.4 and 5.3 Hz, C*H*NH), 3.70 (3H, s, OCH_3), 3.46 (1H, d, *J* = 12.9 Hz, NHC$H^A$$H^B$), 3.38 (1H, d, *J* = 12.9 Hz, NHC$H^A$$H^B$), 2.53 (1H, dd, *J* = 15.4 and 8.4 Hz, C$H^A$$CH^BCO_2$), 2.42 (1H, dd, *J* = 15.4 and 5.3 Hz, CH^AC$H^B$$CO_2$), 2.09 (1H, br. s, N*H*), 1.28 (9H, s, C(CH_3)$_3$); ^{13}C NMR (75 MHz, $CDCl_3$) δ_C = 170.4, 158.8, 150.7, 147.3, 131.8, 129.3, 128.3, 123.8, 113.9, 81.3, 58.5, 55.3, 50.9, 43.7, 28.0; IR (thin film) u_{max} (cm^{-1}): 1722 (s, C=O); HRMS (ESI): *m/z* 409.1777, $C_{21}H_{26}N_2NaO_5$ $[M+Na]^+$ requires 409.1739.

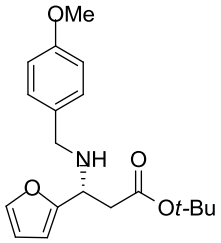
(*R*)-*tert*-Butyl 3-((4-methoxybenzyl)amino)-3-(thiophen-2-yl)propanoate 326f



Preparation from **336f** employing the same method as that used for **326a** afforded the title compound as a colourless oil (0.78 g, 86% yield). $[\alpha]_D^{26}$ +23.5 (*c* 3.4, EtOAc); R_f = 0.33 (EtOAc-petrol, 1:5); ^{1}H NMR (300 MHz, $CDCl_3$) δ_H = 7.19-7.12 (3H, m, C*H*$CHCOCH_3$, C*H*S), 6.89-6.86 (2H, m, C*H*C*H*CS), 6.77 (2H, d, *J* = 8.6 Hz, CHC*H*$COCH_3$), 4.30 (1H, dd, *J* = 8.0 and 5.5 Hz, C*H*NH), 3.72 (3H, s, OCH_3), 3.63 (1H, d, *J* = 12.8 Hz, NHC$H^A$$H^B$), 3.50

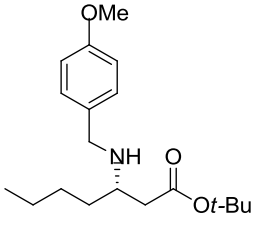
(1H, d, *J* = 12.9 Hz, NHCH^{A}*H*^{B}), 2.64 (1H, dd, *J* = 15.5 Hz, 8.3, C*H*^{A}CH^{B}CO$_2$), 2.56 (1H, dd, *J* = 15.4 and 5.5 Hz, CH^{A}C*H*^{B}CO$_2$), 2.07 (1H, br. s, N*H*), 1.32 (9H, s, C(C*H*$_3$)$_3$); ^{13}C NMR (75 MHz, $CDCl_3$) δ_C = 170.7, 158.6, 147.8, 132.2, 129.5, 126.5, 124.5, 124.4, 113.8, 80.1, 55.3, 54.6, 50.6, 44.6, 28.1; IR (thin film) υ_{max} (cm^{-1}): 1722 (s, C=O); HRMS (ESI): *m/z* 370.1450, $C_{19}H_{25}NNaO_3S$ $[M+Na]^+$ requires 370.1453.

(*R*)-*tert*-Butyl 3-(furan-2-yl)-3-((4-methoxybenzyl)amino)propanoate 326g



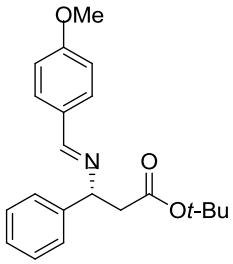
Preparation from **336g** employing the same method as that used for **326g** afforded the title compound as a yellow oil (0.62 g, 73% yield). $[\alpha]_D^{26}$ +57.3 (*c* 3.3, EtOAc)**;** R_f 0.22 (EtOAc-petrol, 1:5); ^{1}H NMR (300 MHz, $CDCl_3$) δ_H = 7.37 (1H, d, *J* = 1.9 Hz, C*H*O), 7.21 (2H, d, *J* = 8.7 Hz, C*H*CHCOCH$_3$), 6.84 (2H, d, *J* = 8.7 Hz, CHC*H*COCH$_3$), 6.32 (1H, dd, *J* = 3.1 and 1.9 Hz, C*H*CHO), 6.20 (1H, d, *J* = 3.1 Hz, C*H*CHCHCO), 4.14 (1H, dd, *J* = 7.7 and 6.4 Hz, C*H*NH), 3.79 (3H, s, OC*H*$_3$), 3.68 (1H, d, *J* = 12.6 Hz, NHC*H*^{A}H^{B}), 3.54 (1H, d, *J* = 12.6 Hz, NHCH^{A}*H*^{B}), 2.18-2.01 (2H, m, C*H*$_2$CO$_2$), 1.96 (1H, br. s, N*H*), 1.40 (9H, s, C(C*H*$_3$)$_3$); ^{13}C NMR (75 MHz, $CDCl_3$) δ_C = 170.7, 158.6, 155.3, 141.7, 132.2, 129.4, 113.7, 110.0, 106.8, 80.7, 55.3, 52.3, 50.5, 41.1, 28.0; IR (thin film) υ_{max} (cm^{-1}): 1725 (s, C=O); HRMS (ESI): *m/z* 354.1691, $C_{19}H_{25}NNaO_4$ $[M+Na]^+$ requires 354.1681.

(*R*)-*tert*-Butyl 3-((4-methoxybenzyl)amino)heptanoate 326h



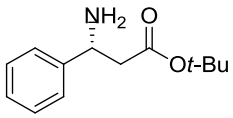
Preparation from **341** employing the same method as that used for **326a** afforded the title compound as a yellow oil (1.2 g, 68% yield). $[\alpha]_D^{26}$ -3.5 (*c* 2.02, CH_2Cl_2); R_f = 0.50 (EtOAc-petrol, 1:5); ^{1}H NMR (300 MHz, $CDCl_3$) δ_H = 7.20 (2H, d, *J* = 8.6 Hz, C*H*CHCOCH$_3$), 6.80 (2H, d, *J* = 8.6 Hz, CHC*H*COCH$_3$), 3.72 (3H, s, OC*H*$_3$), 3.65 (2H, s, NHC*H*$_2$), 2.90 (1H, quintet, *J* = 6.1 Hz, C*H*NH), 2.29 (2H, d, *J* = 6.1 Hz, C*H*$_2$CO$_2$), 1.65 (1H, br. s, N*H*), 1.37 (9H, s, C(C*H*$_3$)$_3$), 1.30-1.14 (6H, m, CH$_3$C*H*$_2$C*H*$_2$C*H*$_2$), 0.82 (3H, t, *J* = 7.2 Hz, C*H*$_3$CH$_2$); ^{13}C NMR (75 MHz, $CDCl_3$) δ_C = 172.4, 158.9, 133.1, 129.7, 114.1, 80.8, 55.7, 54.8, 50.8, 40.8, 34.4, 28.5, 28.3, 23.2, 14.4; IR (thin film) u_{max} (cm^{-1}): 1722 (s, C=O); HRMS (ESI): *m/z* 322.2377, $C_{19}H_{32}NO_3$ [M+H]$^+$ requires 322.2381.

(*R*,*E*)-*tert*-Butyl 3-((4-methoxybenzylidene)amino)-3-phenylpropanoate 353



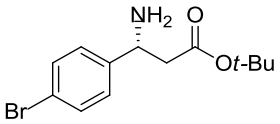
To a solution of **326a** (0.251 g, 0.736 mmol) in MeCN-H_2O (5:1, 9.4 mL) was added CAN (1.72 g, 2.94 mmol). The orange solution was stirred at room temperature for 2 hours and then saturated $NaHCO_{3(aq)}$ (50 mL) was added. The mixture was partitioned between brine and Et_2O and the aqueous layer further extracted with Et_2O (2 x 20 mL). The combined organic extracts were dried over anhydrous $MgSO_4$, filtered and concentrated *in vacuo* to afford the title compound as a yellow oil (0.204 g, 82% yield). $[\alpha]_D^{22}$ -30.0 (*c* 2.9, CH_2Cl_2); ^{1}H NMR (300 MHz, $CDCl_3$) δ_H = 8.36 (1H, s, C*H*=N), 7.76 (2H, d, *J* = 8.5 Hz, C*H*CHCOCH$_3$), 7.50 (2H, d, *J* = 7.7 Hz, Ph), 7.41- 7.36 (2H, m, Ph), 7.32-7.27 (1H, m, Ph), 6.96 (2H, d, *J* = 8.5 Hz, CHC*H*COCH$_3$), 4.82 (1H, dd, *J* = 9.5 and 4.9 Hz, C*H*N=CH), 3.89 (3H, s, OC*H*$_3$), 2.97 (1H, dd, *J* = 15.0 and 1.5 Hz, C*H*^{A}H^B), 2.86 (1H, dd, *J* = 14.9 and 4.8 Hz, CH^A*H*^{B}), 1.39 (9H, s, C(C*H*$_3$)$_3$); ^{13}C NMR (75 MHz, $CDCl_3$) δ_C = 170.7, 161.7, 160.5, 143.1, 130.1, 129.2, 128.5, 127.2, 127.0, 113.9, 80.6, 71.4, 55.4, 44.8, 28.1; IR (thin film) u_{max} (cm^{-1}): 1723 (s, C=O); HRMS (ESI): *m/z* 362.1754, $C_{21}H_{25}NNaO_3$ [M+Na]$^+$ requires 362.1732.

***(R)-tert*-Butyl 3-amino-3-phenylpropanoate[28] 354a**



To a solution of **326a** (0.100 g, 0.293 mmol) in MeCN-H_2O (5:1, 3.8 mL) was added CAN (0.68 g, 1.17 mmol). The orange solution was stirred at room temperature for 2 hours and then saturated $NaHCO_{3(aq)}$ (50 mL) was added. The mixture was partitioned between brine and Et_2O and the aqueous layer further extracted with Et_2O (2 x 20 mL). The combined organic extracts were dried over anhydrous $MgSO_4$, filtered and concentrated *in vacuo*. To the resultant oil dissolved in MeCN-H_2O (5:1, 1.2 mL), was added AcOH (0.23 ml, 3.96 mL) and the mixture stirred at room temperature for 48 hours. The reaction mixture was then diluted with water (5mL) and washed with Et_2O (3 x 10). The aqueous layer was basified with saturated $NaHCO_{3(aq)}$ and extracted with Et_2O (3 x 20). The combined organics were dried over anhydrous $MgSO_4$, filtered and concentrated *in vacuo* to afford the title compound as a yellow oil (0.044 g, 68% yield). $[\alpha]_D^{27}$ +20.0 (*c* 1.2, $CHCl_3$) (lit.[28] $[\alpha]_D^{20}$ +20.0 (*c* 0.71, $CHCl_3$); ^{1}H NMR (300 MHz, $CDCl_3$) δ_H = 7.15-7.33 (5H, m, Ph), 4.31 (1H, t, *J* = 6.9 Hz, C*H*NH), 2.52 (2H, d, *J* = 7.0 Hz, CH_2CO_2), 2.41 (2H, br. s, NH_2), 1.35 (9H, s, C(CH_3)$_3$); ^{13}C NMR (75 MHz, $CDCl_3$) δ_C = 171.4, 128.5, 127.3, 126.3, 80.7, 52.8, 45.4, 28.1; IR (thin film) υ_{max} (cm^{-1}): 3312 (w, N-H), 1725 (s, C=O); HRMS (ESI): *m/z* 222.1500, $C_{13}H_{20}NO_2$ $[M+H]^+$ requires 222.1489.

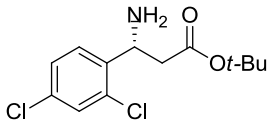
(*R*)-*tert*-Butyl 3-amino-3-(4-bromophenyl)propanoate 354b



Preparation from **326b** employing the same method as that used for **354a** that afforded the title compound as a colourless oil (0.077 g, 56% yield). $[\alpha]_D^{23}$ +21.7 (*c* 0.92, $CDCl_3$); ^{1}H NMR (300 MHz, $CDCl_3$) δ_H = 7.37 (2H, d, *J* = 8.5 Hz, CHC*H*CBr), 7.17 (2H, d, *J* = 8.5 Hz, C*H*CHCBr), 4.29 (1H, br. s, C*H*NH_2), 2.47 (2H, d, *J* = 6.7 Hz, CH_2CO_2), 1.76 (2H, br. s, NH_2), 1.34 (9H, s, C(CH_3)$_3$); ^{13}C NMR (75 MHz, $CDCl_3$) δ_C = 171.0, 143.7, 131.6, 128.2,

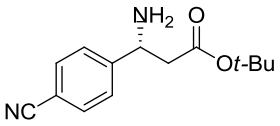
121.0, 81.0, 52.2, 45.1, 28.1; I.R. (thin film) υ_{max} (cm^{-1}): 3662 (m, N-H), 3376 (w, N-H), 1722 (s, C=O); HRMS (ESI): *m/z* 300.0620, $C_{13}H_{19}BrNO_2$ $[M+H]^+$ requires 300.0599.

(*R*)-*tert*-Butyl 3-amino-3-(2,4-dichlorophenyl)propanoate 354c



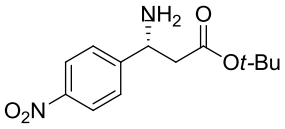
Preparation from **326c** employing the same method as that used for **354a** afforded the title compound as a yellow oil (0.066 g, 68% yield). $[\alpha]_D^{24}$ +37.6 (c 0.85, $CDCl_3$); ^{1}H NMR (300 MHz, $CDCl_3$) δ_H = 7.44 (1H, d, *J* = 8.4 Hz, C*H*CHCCl), 7.20 (1H, d, *J* = 1.8 Hz, CClC*H*CCl), 7.8 (1H, dd, *J* = 8.4 and 1.9 Hz, CClC*H*CH), 4.70 (1H, br. s, C*H*NH_2), 2.60 (1H, dd, *J* = 16.0 and 4.0 Hz, NHCH^AH^B), 2.44 (1H, dd, *J* = 16.0 and 9.0 Hz, NHCH^AH^B), 2.29 (2H, br. s, NH_2), 1.36 (9H, s, C(CH_3)$_3$); ^{13}C NMR (75 MHz, $CDCl_3$) δ_C = 170.8, 133.4, 133.3, 129.4, 128.4, 127.5, 81.2, 48.7, 42.8, 28.1; IR (thin film) υ_{max} (cm^{-1}): 3383 (w, N-H), 1725 (s, C=O); HRMS (ESI): *m/z* 312.0525, $C_{13}H_{17}Cl_2NNaO_2$ $[M+Na]^+$ requires 312.0534.

***(*R*)-tert*-Butyl 3-amino-3-(4-cyanophenyl)propanoate 354d**



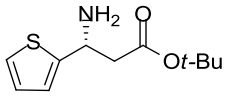
Preparation from **326d** employing the same method as that used for **354a** afforded the title compound as a yellow oil (0.057 g, 51% yield). $[\alpha]_D^{21}$ +16.0 (*c* 0.5, $CDCl_3$); ^{1}H NMR (300 MHz, $CDCl_3$) δ_H = 7.68 (2H, d, *J* = 8.0 Hz, CHC*H*CCN), 7.55 (2H, d, *J* = 8.0 Hz, C*H*CHCCN), 4.50 (1H, br. s, C*H*NH_2), 2.63 (2H, d, *J* = 6.6 Hz, CH_2CO_2), 2.14 (2H, br. s, NH_2), 1.46 (9H, s, C(CH_3)$_3$); ^{13}C NMR (75 MHz, $CDCl_3$) δ_C = 171.6, 132.4, 127.3, 118.8, 111.3, 81.3, 52.5, 44.8, 28.1; I.R. (thin film) υ_{max} (cm^{-1}): 2228 (m, CN), 1723 (s, C=O); HRMS (ESI): *m/z* 247.1448, $C_{14}H_{19}N_2O_2$ $[M+H]^+$ requires 247.1447.

***(*R*)-tert*-Butyl 3-amino-3-(4-nitrophenyl)propanoate 354e**



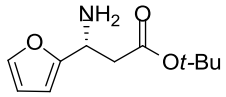
Preparation from **326e** employing the same method as that used for **354a** afforded the title compound as a yellow oil (0.057 g, 65% yield). $[\alpha]_D^{25}$ +9.1 (*c* 0.55, $CDCl_3$); ^{1}H NMR (300 MHz, $CDCl_3$) δ_H = 8.11 (2H, d, *J* = 8.9 Hz, CHC*H*CNO_2), 7.49 (2H, d, *J* = 8.6 Hz, C*H*$CHCNO_2$), 4.42 (1H, br. s, C*H*NH_2), 2.51 (2H, d, *J* = 6.7 Hz, C*H*$_2CO_2$), 1.78 (2H, br. s, N*H*$_2$), 1.34 (9H, s, C(C*H*$_3$)$_3$); ^{13}C NMR (75 MHz, $CDCl_3$) δ_C = 170.6, 152.2, 147.2, 127.4, 123.8, 81.3, 52.3, 45.0, 28.1; IR (thin film) υ_{max} (cm^{-1}): 3382 (w, N-H), 1720 (s, C=O); HRMS (ESI): *m/z* 289.1156, $C_{13}H_{18}N_2NaO_4$ $[M+Na]^+$ requires 289.1164.

(*R*)-*tert*-Butyl 3-amino-3-(thiophen-2-yl)propanoate 354f



Preparation from **326f** employing the same method as that used for **354a** afforded the title compound as a yellow oil (0.048 g, 36% yield). $[\alpha]_D^{25}$ +18.2 (*c* 0.44, $CDCl_3$); ^{1}H NMR (300 MHz, $CDCl_3$) δ_H = 7.27 (1H, dd, *J* = 1.7 and 0.7 Hz, C*H*S), 6.23 (1H, dd, *J* = 3.1 and 1.8 Hz, C*H*CHCS), 6.09 (1H, d, *J* = 3.2 Hz, C*H*CCS), 4.31 (1H, dd, *J* = 8.3 and 4.9 Hz, C*H*NH), 2.68 (1H, dd, *J* = 15.8 and 5.0 Hz, CH^AH^BCO_2), 2.55 (1H, dd, *J* = 15.4 and 8.4 Hz, $CH^ACH^BCO_2$), 2.34 (1H, br. s, N*H*$_2$), 1.3 (9H, s, C(C*H*$_3$)$_3$); ^{13}C NMR (75 MHz, $CDCl_3$) δ_C = 170.8, 157.1, 141.6, 110.1, 104.7, 81.0, 46.8, 42.1, 28.1; IR (thin film) υ_{max} (cm^{-1}): 1727 (s, C=O); HRMS (ESI): *m/z* 228.1078, $C_{11}H_{18}NO_2S$ $[M+H]^+$ requires 228.1058.

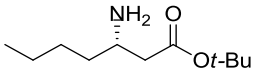
(*R*)-*tert*-Butyl 3-amino-3-(furan-2-yl)propanoate 354g



Preparation from **326g** employing the same method as that used for **354a** afforded the title compound as a pale green oil (0.048 g, 73% yield). $[\alpha]_D^{25}$ +12.4 (*c* 1.05, $CDCl_3$); ^1H NMR (300 MHz, $CDCl_3$) δ_H = 7.12 (1H, dd, *J* = 4.5 and 1.9 Hz, C*H*CHO), 6.89-6.84 (2H, m, C*H*OCC*H*), 4.58 (1H, dd, *J* = 8.4 and 4.9 Hz, C*H*NH_2), 2.65 (1H, dd, *J* = 15.9 and 4.9 Hz, C$H^AH^BCO_2$), 2.57 (1H, dd, *J* = 15.9 and 8.4 Hz, C$H^AH^BCO_2$), 2.22 (2H, br. s, NH_2), 1.36 (9H, s, C(CH_3)$_3$); ^{13}C NMR (75 MHz, $CDCl_3$) δ_C = 170.8, 149.0,126.6, 124.0, 123.2, 81.0, 48.7, 45.6, 28.1; IR (thin film) υ_{max} (cm^{-1}): 3385 (w, N-H), 1723 (s, C=O); HRMS (ESI): *m/z* 212.1286, $C_{11}H_{18}NO_3$ $[M+H]^+$ requires 212.1287.

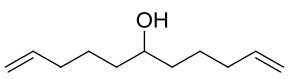
6.5 Procedures and Data for Chapter 5

(*R*)-*tert*-Butyl 3-aminoheptanoate 354h



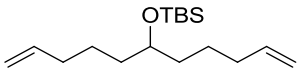
Preparation from **326h** employing the same method as that used for **354a** afforded the title compound as a colourless oil (0.089 g, 60% yield). $[\alpha]_D^{23}$ +33.3 (*c* 1.2, $CDCl_3$); ^1H NMR (300 MHz, $CDCl_3$) δ_H = 3.13-3.00 (1H, m, C*H*NH_2), 2.31 (1H, dd, *J* = 15.6 and 4.0 Hz, C$H^AH^BCO_2$), 2.10 (1H, dd, *J* = 15.6 and 8.8 Hz, C$H^AH^BCO_2$), 1.61 (2H, br. s, NH_2), 1.39 (9H, s, C(CH_3)$_3$), 1.36-1.19 (6H, m, $CH_2CH_2CH_2CH_3$), 0.83 (3H, t, *J* = 6.3 Hz, CH_2CH_3); ^{13}C NMR (75 MHz, $CDCl_3$) δ_C = 172.1, 80.5, 48.4, 43.8, 37.2, 28.2, 28.1, 22.7, 14.0; IR (thin film) υ_{max} (cm^{-1}): 3385 (w, N-H), 1723 (s, C=O); HRMS (ESI): *m/z* 202.1801, $C_{11}H_{24}NO_2$ $[M+H]^+$ requires 202.1807.

Undeca-1,10-dien-6-ol[29] 430



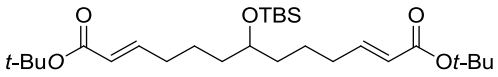
Mg turnings (0.36 g, 15 mmol) were covered with dry THF (4 mL), stirred briskly and 5-bromo-pent-1-ene **2** (2.03 g, 13.6 mmol) was added as a dry THF (11 mL) solution. The addition was kept at a rate sufficient to maintain the reaction at reflux, and reflux was maintained for an hour post addition by heating. The mixture was cooled to 25 °C and ethyl formate (0.47 ml, 5.8 mmol) added dropwise and then heated at reflux for a further hour post-addition. The mixture was cooled to 0 °C and quenched with saturated $NH_4Cl_{(aq)}$ (8 mL) and stirred for 20 minutes, then extracted with Et_2O (3 x 100 mL). The combined organic extracts were washed with saturated $Na_2CO_{3(aq)}$, brine and dried over anhydrous $MgSO_4$, filtered and concentrated *in vacuo*. Purification by silica gel chromatography (EtOAc-petrol, 10:1) afforded the title compound as a yellow oil (0.96 g, 96%). ^{1}H NMR (300 MHz, $CDCl_3$) δ_H = 5.84-5.65 (2H, m, C*H*=CH_2), 5.00-4.82 (4H, m, CH=C*H*$_2$), 3.60-3.49 (1H, m, C*H*OH), 2.10-1.91 (4H, m, C*H*$_2$-CH), 1.55-1.27 (8H, m, CHOH-C*H*$_2$-C*H*$_2$); ^{13}C NMR (75 MHz, $CDCl_3$) δ_C = 138.7, 14.6, 71.7, 36.9, 33.7, 24.9; IR (thin film) υ_{max} (cm^{-1}): 3346 (br., O-H), 1641 (m, C=C); HRMS (ESI): *m/z* 169.1587, $C_{11}H_{21}O$ $[M+H]^+$ requires 169.1592.

tert-Butyldimethyl(undeca-1,10-dien-6-yloxy)silane 431



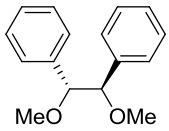
To a stirred solution of **430** (0.4 g, 2.38 mmol) in CH_2Cl_2 (30 mL) were added imidazole (0.486 g, 7.14 mmol), DMAP (0.044 g, 0.36 mmol) and *tert*-butyldimethylsilyl chloride (1.43 g, 9.52 mmol). The mixture was stirred at room temperature and monitored by TLC. After 24 hours saturated $Na_2CO_{3(aq)}$ (10 mL) was added, the mixture was partitioned and the organic extracts were dried over anhydrous $MgSO_4$, filtered and concentrated *in vacuo*. Purification by silica gel chromatography (EtOAc-petrol, 5:1) afforded the title compound as a colourless oil (0.35 g, 52%). ^{1}H NMR (300 MHz, $CDCl_3$) δ_H = 5.77 (2H, ddt, *J* = 17.2, 10.2 and 6.5 Hz, C*H*=CH_2), 5.01-4.87 (4H, m, CH=C*H*$_2$), 3.61 (1H, quintet, *J* = 6.3 Hz, C*H*O-Si), 2.06-1.92 (4H, m, C*H*$_2$-CH=), 1.46-1.28 (8H, m, CHOC*H*$_2$C*H*$_2$), 0.85 (9H, s, Si-C-C*H*$_3$), 0.00 (6H, s, Si-C*H*$_3$); ^{13}C NMR (75 MHz, $CDCl_3$) δ_C = 139.0, 114.3, 72.0, 36.5, 33.9, 25.9, 24.6, 18.2, -4.4; IR (thin film) υ_{max} (cm^{-1}): 1641 (m, C=C); HRMS (ESI): *m/z* 305.2274, $C_{17}H_{34}NaOSi$ $[M+Na]^+$ requires 305.2277.

(2*E*,11*E*)-Di-*tert*-butyl 7-(*tert*-butyldimethylsilyloxy)trideca-2,11-dienedioate 433



To a stirred solution of **431** (3.2 g, 11.3 mmol) in dry CH_2Cl_2 (32 mL) were added *tert*-butyl acrylate **432** (4.9 ml, 33.9 mmol) and Grubbs 2nd generation catalyst (0.096 g, 0.113 mmol). After stirring at room temperature for 48 hours, TLC analysis showed the reaction had gone to completion. The mixture was filtered through a silica plug and washed through with CH_2Cl_2 (500 mL). The filtrates were dried over anhydrous $MgSO_4$, filtered and concentrated *in vacuo* to yield the title compound as a yellow oil (4.80 g, 89%) that did not require further purification. ^{1}H NMR (250 MHz, $CDCl_3$) δ_H = 6.88-6.74 (2H, m, C*H*-CH_2), 5.70 (2H, dt, *J* = 15.5 and 1.5 Hz, C*H*=$CHCH_2$), 3.66-3.56 (1H, m, C*H*O-Si), 2.21-2.04 (4H, m, CH_2-CH), 1.55-1.29 (26H, m, CH_2CH_2CHO, OC(CH_3)$_3$), 0.85 (9H, s, Si-C(CH_3)$_3$), 0.01 (6H, s, Si(CH_3)$_2$); ^{13}C NMR (75 MHz, $CDCl_3$) δ_C = 66.0, 147.7, 123.2, 80.0, 71.6, 36.4, 32.1, 28.4, 25.9, 23.7, 18.1, -4.4; IR (thin film) υ_{max} (cm^{-1}): 1713 (s, C=O), 1653 (m, C=C); HRMS (ESI): *m/z* 483.3518, $C_{27}H_{51}O_5Si$ $[M+H]^+$ requires 483.3506.

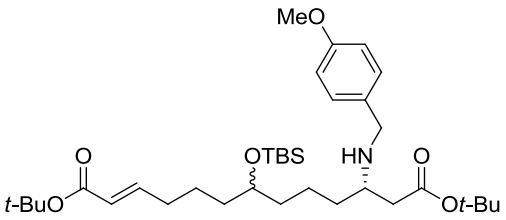
(1*R*,2*R*)-1,2-Dimethoxy-1,2-diphenylethane[30] 303



A stirred suspension of sodium hydride (60% dispersion, 0.467 g, 11.7 mmol) in dry THF (10 mL) was brought to reflux and hydrobenzoin (1.00 g, 4.67 mmol) was subsequently added dropwise. Following the addition the mixture was heated at reflux for a further 30 minutes and then cooled to 0 °C prior to the addition of methyl iodide (15.0 ml, 100 mmol). The reaction was then stirred at room temperature overnight, whereupon TLC analysis showed the reaction had gone to completion. The mixture was cooled to 0 °C and quenched with water (10 mL), extracted with EtOAc (3 x 50 mL), washed with brine, dried over anhydrous $MgSO_4$, filtered and concentrated *in vacuo*. The residue was re-crystallised from petrol to afford the title compound as a white crystalline solid (0.68 g, 60%). $[\alpha]_D^{30}$ -15.1 (*c* 1.8, $CHCl_3$) (lit. value[30] $[\alpha]_D^{25}$ -15.2 (*c* 2.1, $CHCl_3$); ^{1}H NMR (300 MHz,

$CDCl_3$) δ_H = 7.23-7.15 (6H, m, Ph), 7.08-6.98 (4H, m, Ph), 4.34 (2H, s, C*H*OCH_3), 3.30 (6H, s, OC*H*$_3$); ^{13}C NMR (75 MHz, $CDCl_3$) δ_C = 138.2, 127.9, 127.6, 87.7, 57.2; IR (thin film) υ_{max} (cm^{-1}): 1492 (w, C=C), 1455 (w, C=C); HRMS (ESI): *m/z* 265.1188, $C_{16}H_{18}NaO_2$ $[M+Na]^+$ requires 265.1199.

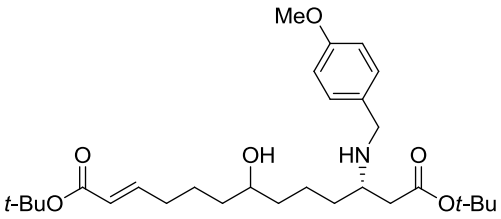
(11*S*,*E*)-Di-*tert*-butyl 7-(*tert*-butyldimethylsilyloxy)-11-(4-methoxybenzylamino)tridec-2-enedioate 434



To a stirred solution of *n*-butyllithium (2.6 M in hexanes, 0.48 ml, 1.24 mmol) in dry toluene (2 mL), cooled to -78 °C, was added amine **325** (0.25 ml, 1.24 mmol) in dry toluene (1 mL) ensuring the temperature was maintained below -60 °C. The mixture was stirred for a further 30 mins at -78 °C and chiral ligand **303** (0.36 g, 1.49 mmol) in dry toluene (2 mL) was added dropwise maintaining the temperature below -60 °C. The mixture was stirred for 30 mins at -78 °C and then added dropwise to a solution of **433** (0.60 g, 1.24 mmol) and chlorotrimethyl-silane (0.78 ml, 6.2 mmol) in dry toluene (3 mL), which was cooled to -78 °C. After being stirred at -78 °C for 5 hours, the mixture was quenched with saturated $NH_4Cl_{(aq)}$ (1.5 mL), allowed to stir-warm to room temperature and partitioned with saturated $Na_2CO_{3(aq)}$ (18 mL). The aqueous phase was further extracted with EtOAc (2 x 50 mL) and the combined organic extracts were washed with brine, dried over anhydrous $MgSO_4$, filtered and concentrated *in vacuo*. Purification by silica gel chromatography (EtOAc-petrol, 20:1 followed by CH_2Cl_2-MeOH, 100:3) afforded the title compound as a yellow oil (0.36 g, 30%). $[\alpha]_D^{26}$ +11.6 (*c* 1.9, $CHCl_3$); ^{1}H NMR (500 MHz, $CDCl_3$) δ_H = 7.23 (2H, d, *J* = 8.2 Hz, MeOCCHC*H*), 6.88-6.75 (3H, m, MeOCC*H*, CH=C*H*CH_2), 5.71 (1H, dt, *J* = 15.4 and 1.9 Hz, C*H*=CHCH_2), 3.76 (3H, s, OC*H*$_3$), 3.69 (2H, s, C*H*$_2$NH), 3.67-3.55 (1H, m, C*H*OSi), 3.00-2.88 (1H, m, C*H*NH), 2.33 (2H, d, *J* = 6.2 Hz, C*H*$_2$C=O), 2.19-2.07 (2H, m, CH=CHC*H*$_2$), 1.71-1.11 (28H, m, C*H*$_2$C*H*$_2$C*H*$_2$CHOC*H*$_2$C*H*$_2$, OCC*H*$_3$), 0.85 (9H, s, SiC(C*H*$_3$)$_3$), 0.00 (6H, s, SiC*H*$_3$); ^{13}C NMR (126 MHz, $CDCl_3$) δ_C = 172.0, 158.6, 147.8, 129.3, 123.1, 113.8, 80.4, 80.00, 71.8, 55.3, 54.4, 50.5, 36.5, 34.6, 32.2, 28.6, 25.9, 23.8, 21.4, 18.1, -4.4; IR (thin film) υ_{max} (cm^{-1}):

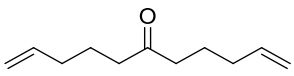
3300 (w, NH), 1718 (s, C=O); HRMS (ESI): *m/z* 642.4180, $C_{35}H_{61}NNaO_6Si$ $[M+Na]^+$ requires 642.4160.

(11*S*,*E*)-Di-*tert*-butyl 7-hydroxy-11-(4-methoxybenzylamino)tridec-2-enedioate 436



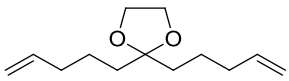
To a stirred solution of **434** (0.68 g, 1.1 mmol) in THF (7 mL) at room temperature was added TBAF (1.0 M in THF, 11 ml, 11 mmol) and benzoic acid (0.54 g, 4.39 mmol). The mixture was heated to 60 °C for 17 hours. After cooling to room temperature water (10 mL) was added and the solution was extracted with EtOAc (3 x 10 mL). The combined organic extracts were washed with saturated $Na_2CO_{3(aq)}$ (3 x 5 mL) and water, dried over anhydrous $MgSO_4$, filtered and concentrated to a yellow oil. Purification by silica gel chromatography (CH_2Cl_2-MeOH, 100:3) afforded the title compound as a colourless oil (0.213 g, 38%). $[\alpha]_D^{25}$ +10.5 (*c* 2.2, CH_2Cl_2); ^{1}H NMR (500 MHz, $CDCl_3$) δ_H = 7.22 (2H, d, *J* = 7.8 Hz, MeOCCHC*H*), 6.89-6.76 (3H, m, MeOCCHC*H*, CH=C*H*CH_2), 5.68-5.67 (1H, m, C*H*=CHCH_2), 3.76 (3H, s, OCH_3), 3.69 (2H, s, CH_2N), 3.60-3.50 (1H, m, C*H*OH), 3.03-2.88 (1H, m, C*H*NH), 2.36 (2H, d, *J* = 5.8 Hz, CH_2CO), 2.22-2.09 (2H, m, CH_2CH=), 1.90 (1H, br. s, O*H*), 1.58-1.30 (28H, m, CH_2CH_2CH_2CHOCH_2CH_2, OC(CH_3)$_3$); ^{13}C NMR (126 MHz, $CDCl_3$) δ_C = 171.9, 166.1, 158.7, 147.6, 129.5, 123.2, 113.8, 80.6, 80.0, 71.2, 55.3, 54.2, 50.3, 40.1, 37.3, 32.0, 28.2, 24.3, 21.7; IR (thin film) u_{max} (cm^{-1}): 1727 (s, C=O); HRMS (ESI): *m/z* 506.3473, $C_{29}H_{48}NO_6$ $[M+H]^+$ requires 506.3482.

Undeca-1,10-dien-6-one 448



A stirred solution of oxalyl chloride (2.82 ml, 33.3 mmol) in dry CH_2Cl_2 (50 mL) was cooled to -60 °C. A solution of DMSO (4.73 ml, 66.6 mmol) in dry CH_2Cl_2 (17 mL) was then added dropwise, keeping the temperature below -60 °C. Taking care to maintain the temperature control, the mixture was stirred for a further 10 minutes and then alcohol **430** (4.0 g, 23.8 mmol), dissolved in dry CH_2Cl_2 (100 mL), was added dropwise. The mixture was stirred at this temperature for 15 minutes and then triethylamine (19.2 ml, 13.8 mmol) in dry CH_2Cl_2 (50 mL) was added dropwise. The suspension was then allowed to stir-warm to room temperature. After quenching with water (200 mL), the aqueous phase was separated and extracted with CH_2Cl_2 (2 x 200 mL). The combined organic extracts were washed with 1% $HCl_{(aq)}$, water, saturated $NaHCO_{3(aq)}$ and water, dried over anhydrous $MgSO_4$, filtered and concentrated to afford the title compound as a yellow oil (3.85 g, 97%). ^{1}H NMR (300 MHz, $CDCl_3$) δ_H = 5.79-5.61 (2H, m, C*H*=CH_2), 4.99-4.88 (4H, m, CH=C*H*$_2$), 2.33 (4H, t, *J* = 7.5 Hz, C*H*$_2$CO), 1.98 (4H, m, C*H*$_2$CH=), 1.6 (4H, m, CH_2C*H*$_2$$CH_2$); ^{13}C NMR (75 MHz, $CDCl_3$) δ_C = 210.9, 138.0, 115.2, 41.9, 33.1, 22.8; IR (thin film) u_{max} (cm^{-1}): 1711 (s, C=O), 1641 (m, C=C); HRMS (ESI): *m/z* 189.1260, $C_{11}H_{18}NaO$ $[M+Na]^+$ requires 189.1255.

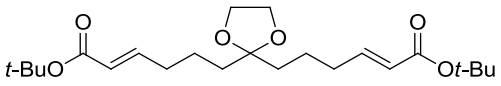
2,2-Di(pent-4-enyl)-1,3-dioxolane 449



To a stirred solution of ketone **448** (3.3 g, 9.9 mmol) in dry toluene was added glycol (3.3 ml, 59.7 mmol) and *p*-toluenesulfonic acid monohydrate (0.379 g, 1.99 mmol). The mixture was heated at reflux for 18 hours, then cooled and concentrated *in vacuo*. The oil was diluted with Et_2O (50 mL), washed successively with saturated $Na_2CO_{3(aq)}$ (20mL), brine (20mL) and water (20mL), dried over anhydrous $MgSO_4$, filtered and concentrated to a yellow oil (4.42g). The oil was taken up in MeOH (40 mL), $NaBH_4$ (0.16 g, 4.19 mmol) was added and the reaction mixture was stirred at room temperature for 18 hours. The mixture was concentrated, partitioned between water (20 mL) and CH_2Cl_2 (20 mL) and the aqueous phase was further extracted with CH_2Cl_2 (2 x 10 mL). The combined organic extracts were washed with water (20 mL), dried over anhydrous $MgSO_4$, filtered and concentrated to a yellow oil. The residue was purified by chromatography on neutral Al_2O_3 (EtOAc-petrol, 1:100) to afford the title compound as a colourless oil (3.04 g, 73%). ^{1}H NMR (300 MHz, $CDCl_3$) δ_H = 5.80-5.65 (2H, m, C*H*=CH_2), 5.00-4.75 (4H, m, CH=C*H*$_2$),

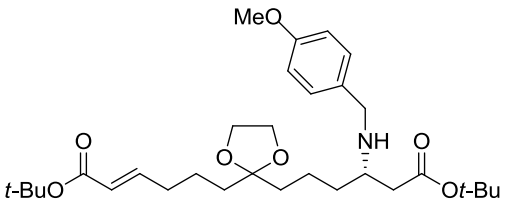
3.90 (4H, s, OCH_2CH_2O), 2.05-1.95 (4H, m, CH_2CH=), 1.60-1.45 (4H, m, CH_2C, 1.45-1.30 (4H, m, CH_2C$H_2$$CH_2$); ^{13}C NMR (75 MHz, $CDCl_3$) δ_C = 138.7, 114.6, 111.7, 64.9, 36.6, 33.9, 23.1; IR (thin film) υ_{max} (cm^{-1}): 1641 (m, C=C); HRMS (ESI): *m/z* 211.1687, $C_{13}H_{23}O_2$ $[M+H]^+$ requires 211.1693.

(2*E*,2'*E*)-*tert*-Butyl 6,6'-(1,3-dioxolane-2,2-diyl)dihex-2-enoate 4



Preparation from **449** employing the same method as that used for **433** furnished a residue that was purified by silica gel chromatography (EtOAc-petrol, 1:30 to 1:9) to afford the title compound as a colourless oil (2.01g, 51%). ^{1}H NMR (300 MHz, $CDCl_3$) δ_H = 6.88 (2H, dt, *J* = 15.6 and 6.9 Hz, C*H*=CHCO), 5.76 (2H, dt, *J* = 15.6 and 1.5 Hz, CH=C*H*CO), 3.95 (4H, s, OCH_2CH_2O), 2.20 (4H, qd, *J* = 6.9 and 1.3 Hz, CHC$H_2$$CH_2$), 1.75-1.45 (26H, m, OC(CH_3)$_3$, CCH_2C$H_2$$CH_2$); ^{13}C NMR (75 MHz, $CDCl_3$) δ_C = 166.5, 147.8, 123.7, 111.7, 80.4, 65.4, 37.2, 32.5, 28.6, 22.7; IR (film/cm^{-1}): 1709 (s, C=O), 1652 (m, C=C); HRMS (ESI): *m/z* 433.2566, $C_{23}H_{38}NaO_6$ $[M+Na]^+$ requires 433.2567.

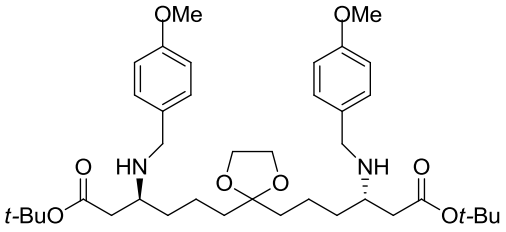
(*S*,*E*)-*tert*-Butyl 6-(2-(6-*tert*-butoxy-4-(4-methoxybenzylamino)-6-oxohexyl)-1,3-dioxolan-2-yl)hex-2-enoate 5



Preparation from **4** employing the same method as that used for **434** furnished a residue that was purified by silica gel chromatography (CH_2Cl_2-MeOH saturated with $NH_{3(g)}$, 100:0.5 to 100:1.5) to afford the title compound as a yellow oil (0.75 g, 40%). $[\alpha]_D^{25}$ -7.5 (*c* 0.5, CH_2Cl_2); R_f 0.19 (CH_2Cl_2-MeOH, saturated with NH_3, 100:1); ^{1}H NMR (500 MHz, $CDCl_3$) δ_H = 7.18 (2H, d, *J* = 8.1 Hz, MeOCCHC*H*), 6.83-6.70 (3H, m, MeOCC*H*, C*H*=CHCO), 5.67 (1H, dt, *J* = 15.5 and 1.6 Hz, CH=C*H*CO), 3.84 (4H, s, OCH_2CH_2O),

3.72 (3H, s, OCH_3), 3.66 (2H, s, NHCH_2), 2.92 (H, q, *J* = 6.1 Hz, C*H*NH), 2.32 (2H, d, *J* = 6.1 Hz, CHNCH_2CO_2), 2.10-1.98 (2H, m, CH_2CH=CH), 1.62-1.24 (28H, m, C$H_2CH_2CH_2C(OCH_2)_2CH_2CH_2$, OCC$H_3$); ^{13}C NMR (126 MHz, $CDCl_3$) δ_C = 172.3, 166.5, 159.1, 147.9, 129.8, 123.6, 114.2, 111.7, 81.0, 80.4, 65.4, 55.7, 54.7, 50.7, 37.7, 37.1, 32.5, 28.6, 28.4, 22.7, 20.4; IR (thin film) υ_{max} (cm^{-1}): 1714 (s, C=O), 1611 (w, C=C); HRMS (ESI): *m/z* 548.3563, $C_{31}H_{50}NO_7$ $[M+H]^+$ requires 548.3587.

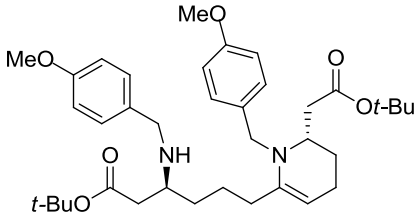
(3*S*,3'*S*)-Di-*tert*-butyl 6,6'-(1,3-dioxolane-2,2-diyl)*bis*(3-((4-methoxybenzyl)amino) hexanoate) 453



To a stirred solution of *n*-butyllithium (2.6 M in hexanes, 2.44 ml, 6.1 mmol) in dry toluene (12 mL) cooled to -78 °C was added amine **325** (1.28 g, 6.1 mmol) in dry toluene (7 mL) ensuring the temperature was maintained below -60 °C. The mixture was stirred for 30 minutes at -78 °C and then chiral ligand **303** (1.66 g, 6.83 mmol) in dry toluene (7 mL) was added dropwise maintaining the temperature below -60 °C. The mixture was then stirred for 30 minutes at -78 °C, followed by dropwise addition of **4** (1 g, 2.44 mmol) and chlorotrimethyl- silane (3.08 ml, 24.4 mmol) in dry toluene (25 mL). After stirring at -78 °C for 5 hours the mixture was quenched with saturated $NH_4Cl_{(aq)}$ (13 mL), allowed to stir-warm to room temperature and partitioned with saturated $NaHCO_{3(aq)}$ (100 mL). The aqueous phase was further extracted with EtOAc (2 x 100 mL) and the combined organic extracts were washed with brine, dried over anhydrous $MgSO_4$, filtered and concentrated *in vacuo*. Purification by silica gel chromatography (CH_2Cl_2-MeOH saturated with NH_3, 100:0.5 to 100:4) afforded the title compound as a yellow oil (0.82 g, 49% yield). $[\alpha]_D^{26}$ +55.0 (*c* 0.49, CH_2Cl_2); R_f = 0.22 (CH_2Cl_2–MeOH saturated with NH_3, 100:4); ^{1}H NMR (500 MHz, $CDCl_3$) δ_H = 7.25 (4H, d, *J* = 8.6 Hz, C*H*CHCOCH$_3$), 6.84 (4H, d, *J* = 8.7 Hz, CHC*H*COCH$_3$), 3.91 (4H, s, OCH_2CH_2O), 3.78 (6H, s, OCH_3), 3.71 (4H, s, NHCH_2), 2.37 (4H, d, *J* = 5.9 Hz, CH_2C=O), 1.82 (2H, br. s, N*H*), 1.63-1.53 (4H, m, C$H_2CO_2CH_2$), 1.50-1.32 (8H, m, CH_2), 1.44 (18H, s, OC(CH_3)$_3$); ^{13}C NMR (126 MHz, $CDCl_3$) δ_C = 172.0, 158.6, 132.6, 129.4, 113.8, 111.4, 80.4, 65.9, 65.0, 55.3, 54.3, 50.4, 40.3, 37.3, 34.5,

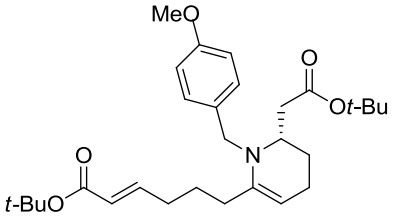
28.2, 20.0, 15.3; IR (thin film) υ_{max} (cm^{-1}): 1721 (s, C=O); HRMS (ESI): *m/z* 685.4446, $C_{39}H_{61}N_2O_8$ $[M+H]^+$ requires 685.4428.

(*S*)-*tert*-Butyl 6-((*S*)-6-(2-(*tert*-butoxy)-2-oxoethyl)-1-(4-methoxybenzyl)-1,4,5,6-tetrahydropyridin-2-yl)-3-((4-methoxybenzyl)amino)hexanoate 453



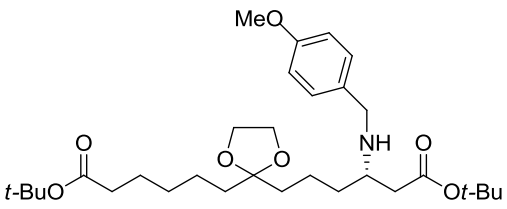
To a solution of *bis*-amine **452** (0.115 g, 0.168 mmol) in acetone (7 mL) was added *para*-toluenesulfonic acid monohydrate (0.048 g, 0.252 mmol) and the mixture was stirred at room temperature whilst being monitored by TLC. After 4 hours the reaction mixture was concentrated *in vacuo*, diluted with CH_2Cl_2 (30 mL), washed with saturated $NaHCO_{3(aq)}$ (3 x 10 mL), dried over anhydrous $MgSO_4$, filtered and concentrated *in vacuo* to afford the title compound as an orange oil requiring no further purification (0.089 g, 85% yield). $[\alpha]_D^{27}$ +5.2 (*c* 1.1, CH_2Cl_2); ^{1}H NMR (500 MHz, $CDCl_3$) δ_H = 7.32 (2H, d, *J* = 8.4 Hz, C*H*CHCOCH$_3$), 7.24 (2H, d, *J* = 8.6 Hz, C*H*CHCOCH$_3$), 6.95-6.87 (4H, m, CHC*H*COCH$_3$), 4.52 (1H, br. s, N=C*H*), 4.20 (1H, d, *J* = 15.8 Hz, NC*H*^{A}H^B), 3.98 (1H, d, *J* = 15.7 Hz, NCH^A*H*^{B}), 3.96 (6H, s, OC*H*$_3$), 3.79 (2H, s, C*H*$_2$NH), 3.57 (1H, br. s, NC=C*H*), 3.06 (1H, br. s, C*H*NH), 2.46 (4H, br. s, C*H*$_2$C=O), 2.27-2.22 (2H, m, NCHC*H*$_2$), 2.05-1.92 (3H, m, NC=CHC*H*$_2$), 1.82-1.45 (6H, m, C*H*$_2$), 1.51 (9H, s, OC(C*H*$_3$)$_3$), 1.50 (9H, s, OC(C*H*$_3$)$_3$); ^{13}C NMR (126 MHz, $CDCl_3$) δ_C = 172.0, 171.9, 158.9, 158.6, 141.5, 132.0, 129.3, 129.6, 129.3, 114.1, 113.9, 98.7, 80.4, 78.0, 55.3, 55.2, 54.2, 54.1, 54.0, 53.4, 40.4, 40.2, 34.0, 33.8, 28.2, 28.0, 24.2, 19.9 ; IR (thin film) υ_{max} (cm^{-1}): 1725 (s, C=O); HRMS (ESI): *m/z* 623.4090, $C_{37}H_{55}N_2O_6$ $[M+H]^+$ requires 623.4060.

(*S*,*E*)-*tert*-Butyl 6-(6-(2-(*tert*-butoxy)-2-oxoethyl)-1-(4-methoxybenzyl)-1,4,5,6-tetrahydropyridin-2-yl)hex-2-enoate 454



Preparation from **5** employing the same method as that used for **453** afforded the title compound as a yellow oil requiring no further purification (0.23 g, 95%). $[\alpha]_D^{25}$ -14.9 (*c* 0.91, CH_2Cl_2); R_f = 0.35 (CH_2Cl_2- MeOH saturated with NH_3, 100:1.5); ^{1}H NMR (500 MHz, toluene-d_8) δ_H = 7.14 (2H, d, *J* = 7.9 Hz, C*H*CHCOCH$_3$), 6.98-7.09 (1H, m, CO_2CH=C*H*), 6.75 (2H, d, *J* = 7.9 Hz, CHC*H*COCH$_3$), 5.90 (1H, d, *J* = 15.7 Hz, CO_2C*H*=CH), 4.51 (1H, br. s, NC=C*H*), 4.13 (1H, d, *J* = 15.2 Hz, NCH^AH^B), 3.92 (1H, d, *J* = 15.2 Hz, NCH^A*H*^{B}), 3.71-3.65 (1H, m, C*H*N), 3.36 (3H, s, OCH_3), 2.47 (1H, dd, *J* = 15.0 and 9.4 Hz, CH^AH^BC=O), 2.41-2.37 (1H, m, C=CCH^AH^B), 1.97 (1H, dd, *J* = 14.9 and 4.8 Hz, CH^A*H*^{B}C=O), 1.94-1.81 (4H, m, NC=CHCH^A*H*^{B}, C=CCH^A*H*^{B}CH$_2$C*H*$_2$), 1.47 (9H, s, OC(CH_3)$_3$), 1.46 (9H, s, OC(CH_3)$_3$), 1.44-1.23 (4H, m, NC=CCH$_2$C*H*$_2$, =CHCH$_2$C*H*$_2$); ^{13}C NMR (126 MHz, toluene-d_8) δ_C = 171.0, 165.2, 158.9, 147.6, 140.5, 131.7, 128.8, 124.9, 113.6, 99.6, 79.1, 78.9, 54.3, 53.8, 52.9, 37.8, 33.4, 31.1, 27.7, 27.6, 26.7, 23.7, 19.5; IR (thin film) υ_{max} (cm^{-1}): 3220 (w, NH), 1711 (s, C=O); HRMS (ESI): *m/z* 486.3227, $C_{29}H_{44}NO_5$ $[M+H]^+$ requires 486.3219.

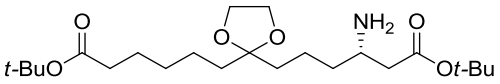
(*S*)-*tert*-Butyl 6-(2-(6-(*tert*-butoxy)-6-oxohexyl)-1,3-dioxolan-2-yl)-3-((4-methoxybenzyl)amino)hexanoate 456



To a solution of **5** (0.5 g, 0.913 mmol) in MeOH (5 mL) was added $CoCl_2.6H_2O$ (2.20 mg, 0.0091 mmol) and the mixture stirred at room temperature for 30 minutes. After cooling

the mixture to 0 °C, $NaBH_4$ (0.138 g, 3.65 mmol) was added portionwise and the reaction mixture was stirred at room temperature for 36 hours. After cooling the reaction mixture to 0 °C, $NaBH_4$ (0.138 g, 3.65 mmol) was again added portionwise and the reaction mixture was stirred at room temperature for a further 24 hours. After cooling the reaction mixture to 0 °C, water (20 mL) was added and then the methanol was removed *in vacuo*.The remaining aqueous solution was extracted with EtOAc (3 x 20 mL). The combined organic extracts were dried over anhydrous $MgSO_4$, filtered and concentrated *in vacuo* to afford the title compound as a yellow oil that required no further purification (0.48 g, 96% yield). $[\alpha]_D^{25}$ +13.7 (*c* 0.95, $CHCl_3$); R_f 0.19 (CH_2Cl_2- MeOH saturated with NH_3, 100:1); ^{1}H NMR (500 MHz, $CDCl_3$) δ_H = 7.18 (2H, d, *J* = 8.7 Hz, C*H*CHCOCH$_3$), 6.78 (2H, d, *J* = 8.7 Hz, CHC*H*COCH$_3$), 3.84 (4H, s, OC*H*$_2$C*H*$_2$O), 3.72 (3H, s, OC*H*$_3$), 3.64 (2H, s, C*H*$_2$NH), 2.95-2.85 (1H, m, C*H*NH), 2.30 (2H, d, *J* = 6.2 Hz, NHCHC*H*$_2$C=O), 2.13 (2H, t, *J* = 7.5 Hz, C*H*$_2$C=O), 1.59-1.45 (4H, m, C*H*$_2$CO$_2$C*H*$_2$), 1.37 (18H, s, OC(C*H*$_3$)$_3$), 1.37-1.21 (10H, m, C*H*$_2$); ^{13}C NMR (126 MHz, $CDCl_3$) δ_C = 173.2, 172.0, 158.6, 132.7, 129.3, 113.8, 111.6, 80.4, 79.9, 64.9, 55.3, 54.3, 50.4, 40.4, 37.2, 37.1, 35.5, 34.6, 29.4, 28.1, 25.1, 23.5, 20.0; IR (thin film) u_{max} (cm^{-1}): 1718 (s, C=O); HRMS (ESI): *m/z* 572.3563, $C_{31}H_{51}NNaO_7$ $[M+Na]^+$ requires 572.3563.

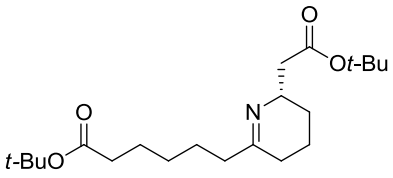
(*S*)-*tert*-Butyl 3-amino-6-(2-(6-(*tert*-butoxy)-6-oxohexyl)-1,3-dioxolan-2-yl)hexanoate[31] 457



To a solution of **456** (0.66 g, 1.20 mmol) in dry MeOH (18 mL) was added 10% $Pd(OH)_2$/C (0.12 g, 0.09 mmol)). The reaction vessel was then purged with H_2 (x3) and a 760 Torr H_2 pressure was maintained for 12 hours. The suspension was filtered through celite, eluting with fresh MeOH (30 mL) and then concentrated *in vacuo* to afford a colourless oil that required no further purification (0.35 g, 68% yield). $[\alpha]_D^{25}$ +7.0 (*c* 10.0, $CDCl_3$); ^{1}H NMR (500 MHz, $CDCl_3$) δ_H = 3.85 (4H, s, OC*H*$_2$C*H*$_2$O), 3.13-3.02 (1H, m, C*H*NH$_2$), 2.32 (1H, dd, *J* = 15.6 and 3.9 Hz, CHNH$_2$C*H*^{A}H^B), 2.13 (2H, t, *J* = 7.4 Hz, C=OC*H*$_2$), 2.10 (1H, dd, *J* = 15.6 and 8.9 Hz, CHNH$_2$CH^A*H*^{B}), 1.59 (2H, br. s, N*H*$_2$), 1.57-1.46 (6H, m, C=OCH$_2$C*H*$_2$, C*H*$_2$CO$_2$C*H*$_2$), 1.39 (9H, s, C(C*H*$_3$)$_3$), 1.37 (9H, s, C(C*H*$_3$)$_3$), 1.35-1.17 (8H, m, C*H*$_2$); ^{13}C NMR (126 MHz, $CDCl_3$) δ_C = 173.2, 172.0, 111.5, 80.5, 79.9, 64.9, 49.4,

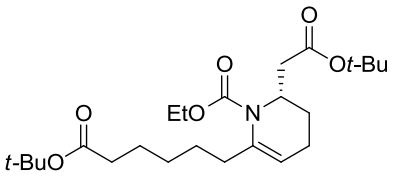
43.7, 37.7, 37.1, 35.5, 29.4, 28.2, 25.1, 23.5, 20.3; IR (thin film) υ_{max} (cm^{-1}): 1726 (s, C=O); HRMS (ESI): *m/z* 452.2978, $C_{23}H_{43}NNaO_6$ $[M+Na]^+$ requires 452.2988.

(*S*)-*tert*-Butyl-6-(6-(2-(*tert*-butoxy)-2-oxoethyl)-3,4,5,6-tetrahydropyridin-2-yl) hexanoate 458



Preparation from **457** employing the same method as that used for **453** afforded the title compound as a yellow oil requiring no further purification (0.37 g, 85%). $[\alpha]_D^{27}$ -6.33 (*c* 1.58, CH_2Cl_2); ^{1}H NMR (500 MHz, $CDCl_3$) δ_H = 3.83 (1H, m, C=NC*H*), 2.66 (1H, dd, *J* = 14.6 and 6.9 Hz, NCHCH^AH^{B}C=O), 2.27 (1H, dd, *J* = 14.5 and 7.9 Hz, NCHCH^AH^BC=O), 2.15-2.11 (2H, m, C=OCH_2), 1.95 (2H, td, *J* = 7.5 and 1.9 Hz, CH_2CH_2C=N), 1.71-1.48 (6H, m, CH_2), 1.46 (9H, s, C(CH_3)$_3$), 1.42 (9H, s, C(CH_3)$_3$), 1.36-1.28 (2H, m, CH_2), 1.27-1.21 (2H, m, CH_2), 1.05-0.91 (2H, m, CH_2); ^{13}C NMR (126 MHz, $CDCl_3$) δ_C = 172.0, 170.8, 168.1, 78.9, 78.8, 55.2, 44.0, 39.9, 35.2, 28.8, 28.5, 27.9, 27.8, 27.2, 25.5, 25.0, 18.9; IR (thin film) υ_{max} (cm^{-1}): 1710 (s, C=O); HRMS (ESI): *m/z* 390.2644, $C_{21}H_{37}NNaO_4$ $[M+Na]^+$ requires 390.2620.

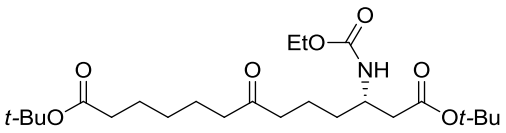
(*S*)-Ethyl 2-(2-(*tert*-butoxy)-2-oxoethyl)-6-(6-(*tert*-butoxy)-6-oxohexyl)-3,4-dihydro-pyridine-1(2H)-carboxylate 493



A solution of **458** (66 mg, 0.180 mmol) in dry toluene (1.6 mL) was heated to 50 °C before the dropwise addition of diethyl pyrocarbonate (48 µl, 0.324 mmol). The reaction mixture was kept at 50 °C for a further 7 hours, then cooled to room temperature and

concentrated *in vacuo*. The residue was purified by silica gel chromatography (EtOAc-petrol, 1:10) to afford the title compound as a yellow oil (57 mg, 72% yield). $[\alpha]_D^{25}$ -25.7 (*c* 0.07, CH_2Cl_2); R_f = 0.42 (EtOAc-petrol, 1:10); ^{1}H NMR (300 MHz, $CDCl_3$) δ_H = 5.16-5.09 (1H, m, C*H*N), 4.79 (1H, br. s, C=C*H*), 4.09 (2H, q, *J* = 7.5 Hz, OCH_2CH$_3$), 2.48 (1H, dd, *J* = 11.1 and 4.6 Hz, NCHCH^AH^{B}C=O), 2.19 (1H, dd, *J* = 11.15.8 Hz, NCHCH^AH^BC=O), 2.18-2.08 (4H, m, O=CCH_2, NCCH_2), 1.8 (2H, br. s, C=CHCH_2), 1.69-1.61 (3H, m, CH_2CH$_2$CNCHCH^AH^B), 1.55-1.29 (5H, m, NCHCH^AH^B, O=CCH$_2$CH_2CH_2), 1.44 (9H, s, O=CO(CH_3)$_3$), 1.40 (9H, s, O=CO(CH_3)$_3$), 1.08 (3H, t, *J* = 7.5 Hz, OCH$_2$CH_3); ^{13}C NMR (75 MHz, $CDCl_3$) δ_C = 172.7, 170.6, 154.4, 138.3, 111.2, 80.2, 79.5, 61.4, 49.9, 37.1, 36.3, 36.0, 29.8, 28.5, 28.4, 26.6, 25.8, 25.6, 19.9, 15.0; I.R. (thin film) υ_{max} (cm^{-1}): 3351 (br., N-H), 1724 (s, C=O); HRMS (ESI): *m/z* 440.3045, $C_{24}H_{42}NO_6$ [M+H]$^+$ requires 440.3012.

(*S*)-Di-*tert*-butyl 3-((ethoxycarbonyl)amino)-7-oxotridecanedioate 494



In a sealed microwave tube, diethyl pyrocarbonate (14 µl, 0.0925 mmol) was added to a solution of **458** (17 mg, 0.0463 mmol) in dry ethanol (0.5 mL). The reaction mixture was then heated at 130 °C for 30 minutes using microwave irradiation. The resultant yellow solution was cooled to room temperature and concentrated *in vacuo*. The residue was then purified by silica gel chromatography (EtOAc-petrol, 1.5:10 to 3.5:10) to afford the title compound as a yellow oil (11 mg, 54 % yield). $[\alpha]_D^{25}$ -25.7 (*c* 0.07, CH_2Cl_2); ^{1}H NMR (300 MHz, $CDCl_3$) δ_H = 4.91 (1H, br. s, N*H*), 4.10 (2H, q, *J* = 7.2 Hz, OCH_2CH$_3$), 4.01 (1H, m, C*H*N) 2.22 (2H, d, *J* = 5.7 Hz,CHNCH_2), 2.11 (4H, m, CH_2COCH_2), 1.95 (2H, t, *J* = 7.4 Hz, O=CCH_2CH$_2$), 1.50-1.31 (8H, m, 4xCH_2), 1.45 (9H, s, O=CO(CH_3)$_3$), 1.41 (9H, s, O=CO(CH_3)$_3$), 1.19-1.10 (2H, m, CH_2), 1.10 (3H, t, *J* = 7.2 Hz, OCH$_2$CH_3); ^{13}C NMR (75 MHz, $CDCl_3$) δ_C = 207.1, 171.6, 169.9, 155.5, 79.3, 78.2, 59.5, 47.1, 41.3, 40.7, 39.5, 34.4, 33.2, 29.3, 28.0, 27.1, 27.0, 24.2, 22.6, 13.8; IR (thin film) υ_{max} (cm^{-1}): 3362 (br., N-H), 1713 (s, C=O); HRMS (ESI): *m/z* 458.3154, $C_{24}H_{44}NO_7$ [M+H]$^+$ requires 458.3118.

6.6 References

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